(54) Title: COMBINATION THERAPY FOR CONDITIONS LEADING TO BONE LOSS

Combination treatment with OPG-Fc and IL-1ra or s-TNF-R1 on adjuvant arthritis in male Lewis rats (AdA-20, D9-D15)

Legend:
- NT
- AdA
- Vehicle
- 0.2 mg/kg/w IL-1ra
- 1.0 mg/kg/w IL-1ra
- 0.25 mg/kg s-TNF-R1
- 1.0 mg/kg s-TNF-R1
- 0.03 mg/kg OPG-Fc
- 0.2 mg/kg/w IL-1ra + 0.03 mg/kg OPG-Fc
- 1.0 mg/kg/w IL-1ra + 0.03 mg/kg OPG-Fc
- 0.25 mg/kg s-TNF-R1 + 0.03 mg/kg OPG-Fc
- 1.0 mg/kg s-TNF-R1 + 0.03 mg/kg OPG-Fc
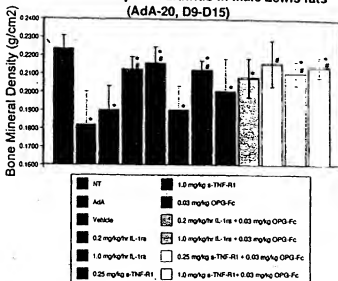
Paws from rats with adjuvant arthritis induced by 0.5mg mycobacteria in oil were analyzed by DEXA for BMD. Evaluation of BMD, a 29mm X 25mm was centered at the tibiotarsal region. (expt AdA-20 5/99, Amgen rbr#22957 p68).
* compared to normal, # compared to vehicle
P < 0.05 Mann-Whitney U test.

(57) Abstract: The present invention discloses a novel secreted polypeptide, termed osteoprotegerin, which is a member of the tumor necrosis factor receptor superfamily and is involved in the regulation of bone metabolism. Also disclosed are nucleic acids encoding osteoprotegerin, recombinant vectors and host cells for expression, antibodies which bind OPG, and pharmaceutical compositions. The polypeptides are used to treat bone diseases characterized by increased resorption such as osteoporosis. Methods of treatment are described using the polypeptides in conjunction with various agents, including IL-1 inhibitors, TNF-α inhibitors, and serine protease inhibitors.

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COMBINATION THERAPY FOR
CONDITIONS LEADING TO BONE LOSS

Cross-Reference to Related Applications

5   This application is a continuation-in-part of U.S.
Ser. No. 09/350,670 filed July 9, 1999, which is a
continuation-in-part (CIP) of U.S. Ser. No. 08/706,945,
filed on September 3, 1996, which in turn is a CIP of
U.S. Ser. No. 08/577,788, filed December 22, 1995. Each
10   of the foregoing applications is hereby incorporated by
reference.

Field of the Invention

The invention relates generally to polypeptides
involved in the regulation of bone metabolism. More
15   particularly, the invention relates to a novel
polypeptide, termed osteoprotegerin, which is a member
of the tumor necrosis factor receptor superfamily. The
polypeptide is used to treat bone diseases
characterized by increased bone loss such as
20   osteoporosis and arthritis.

Background of the Invention

Polypeptide growth factors and cytokines are
secreted factors which signal a wide variety of changes
in cell growth, differentiation, and metabolism, by
25   specifically binding to discrete, surface bound
receptors. As a class of proteins, receptors vary in
their structure and mode of signal transduction. They
are characterized by having an extracellular domain
that is involved in ligand binding, and cytoplasmic
30   domain which transmits an appropriate intracellular
signal. Receptor expression patterns ultimately
determine which cells will respond to a given ligand,
while the structure of a given receptor dictates the
cellular response induced by ligand binding. Receptors
35   have been shown to transmit intracellular signals via

their cytoplasmic domains by activating protein
tyrosine, or protein serine/threonine phosphorylation
(e.g., platelet derived growth factor receptor (PDGFR)
or transforming growth factor-β receptor-I (TGFβR-I),

5   by stimulating G-protein activation (e.g., β-adrenergic
receptor), and by modulating associations with
cytoplasmic signal transducing proteins (e.g., TNFR-I
and Fas/APO) (Heldin, Cell 80, 213-223 (1995)).

      The tumor necrosis factor receptor (TNFR)
10   superfamily is a group of type I transmembrane proteins
which share a conserved cysteine-rich motif which is
repeated three to six times in the extracellular domain
(Smith, et al. Cell 76, 953-962 (1994)). Collectively,
these repeat units form the ligand binding domains of
15   these receptors (Chen et al., Chemistry 270, 2874-2878
(1995)). The ligands for these receptors are a
structurally related group of proteins homologous to
TNFα. (Goeddel et al. Cold Spring Harbor Symp. Quart.
Biol. 51, 597-609 (1986); Nagata et al. Science 267,
20   1449-1456 (1995)). TNFα binds to distinct, but closely
related receptors, TNFR-I and TNFR-II. TNFα produces a
variety of biological responses in receptor bearing
cells, including, proliferation, differentiation, and
cytoxicity and apoptosis (Beutler et al. Ann. Rev.
25   Biochem. 57, 505-518 (1988)).

      TNFα is believed to mediate acute and chronic
inflammatory responses (Beutler et al. Ann. Rev.
Biochem. 57, 505-508 (1988)). Systemic delivery of TNFα
induces toxic shock and widespread tissue necrosis.
30   Because of this, TNFα may be responsible for the severe
morbidity and mortality associated with a variety of
infectious diseases, including sepsis. Mutations in
FasL, the ligand for the TNFR-related receptor Fas/APO
(Suda et al. Cell 75, 1169-1178 (1993)), is associated

with autoimmunity (Fisher et al. Cell 81, 935-946
(1995)), while overproduction of FasL may be implicated
in drug-induced hepatitis. Thus, ligands to the various
TNFR-related proteins often mediate the serious effects
5   of many disease states, which suggests that agents that
neutralize the activity of these ligands would have
therapeutic value. Soluble TNFR-I receptors, and
antibodies that bind TNFα, have been tested for their
ability to neutralize systemic TNFα (Loetscher et al.
10   Cancer Cells 3(6), 221-226 (1991)). A naturally
occurring form of a secreted TNFR-I mRNA was cloned,
and its product tested for its ability to neutralize
TNFα activity in vitro and in vivo (Kohno et al. PNAS
USA 87, 8331-8335 (1990)). The ability of this protein
15   to neutralize TNFα suggests that soluble TNF receptors
function to bind and clear TNF thereby blocking the
cytotoxic effects on TNFR- bearing cells.

An object of the invention is to identify new
members of the TNFR superfamily. It is anticipated that
20   new family members may be transmembrane proteins or
soluble forms thereof comprising extracellular domains
and lacking transmembrane and cytoplasmic domains. We
have identified a new member of the TNFR superfamily
which encodes a secreted protein that is closely
25   related to TNFR-II. By analogy to soluble TNFR-II, the
TNFR-II related protein may negatively regulate the
activity of its ligand, and thus may be useful in the
treatment of certain human diseases.

A further object of this invention is new methods
30   of treatment of inflammatory diseases and medical
conditions.

Summary of the Invention

A novel member of the tumor necrosis factor
receptor (TNFR) superfamily has been identified from a
35   fetal rat intestinal cDNA library. A full-length cDNA

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clone was obtained and sequenced. Expression of the rat
cDNA in a transgenic mouse revealed a marked increase
in bone density, particularly in long bones, pelvic
bone and vertebrae. The polypeptide encoded by the cDNA
5   is termed Osteoprotegerin (OPG) and plays a role in
promoting bone accumulation.

      The invention provides for nucleic acids encoding
a polypeptide having at least one of the biological
activities of OPG. Nucleic acids which hybridize to
10   nucleic acids encoding mouse, rat or human OPG as shown
in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:
122), and 9C-9D (SEQ ID NO: 124) are also provided.
Preferably, OPG is mammalian OPG and more preferably is
human OPG. Recombinant vectors and host cells
15   expressing OPG are also encompassed as are methods of
producing recombinant OPG. Antibodies or fragments
thereof which specifically bind the polypeptide are
also disclosed.

      Methods of treating bone diseases are also
20   provided by the invention. The polypeptides are useful
for preventing bone resorption and may be used to treat
any condition resulting in bone loss such as
osteoporosis, hypercalcemia, Paget's disease of bone,
and bone loss due to rheumatoid arthritis or
25   osteomyelitis, and the like. Bone diseases may also be
treated with anti-sense or gene therapy using nucleic
acids of the invention. Pharmaceutical compositions
comprising OPG nucleic acids and polypeptides are also
encompassed.

30   The invention relates further to treatment of
diseases using combination therapy. In particular, the
novel polypeptides described herein may be used in
conjunction with bone morphogenic proteins BMP-1
through BMP-12; TGF-β and TGF-β family members; IL-1
35   inhibitors; TNF-α inhibitors; parathyroid hormone and

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analogs thereof; parathyroid related protein and
analogs thereof; E series prostaglandins;
bisphosphonates; bone-enhancing minerals; NSAIDs;
immunosuppressants; serine protease inhibitors; IL-6
5   inhibitors; IL-8 inhibitors (e.g., antibodies to IL-8);
IL-18 inhibitors; ICE modulators; FGF-1 to FGF-10; FGF
modulators; PAF antagonists; KGF, KGF-related
molecules, or KGF modulators; MMP modulators; NOS
modulators; modulators of glucocorticoid receptor;
10   modulators of glutamate receptor; modulators of LPS
levels; and noradrenaline and modulators and mimetics
thereof.

Description of the Figures

     Figure 1. A. FASTA analysis of novel EST LORF.
15   Shown is the deduced FRI-1 amino acid sequence aligned
to the human TNFR-II sequence. B. Profile analysis of
the novel EST LORF shown is the deduced FRI-1 amino
acid sequence aligned to the TNFR-profile. C.
Structural view of TNFR superfamily indicating region
20   which is homologous to the novel FRI-1.

     Figure 2. Structure and sequence of full length
rat OPG gene, a novel member of the TNFR superfamily.
A. Map of pMOB-B1.1 insert. Box indicates position of
LORF within the cDNA sequence (bold line). Black box
25   indicates signal peptide, and gray ellipses indicate
position of cysteine-rich repeat sequences. B, C.
Nucleic acid and protein sequence of the Rat OPG cDNA.
The predicted signal peptide is underlined, and
potential sites of N-linked glycosylation are indicated
30   in bold, underlined letters. D, E. Pileup sequence
comparison (Wisconsin GCG Package, Version 8.1) of OPG
with other members of the TNFR superfamily, fas (SEQ ID
NO:128); tnfr1 (SEQ ID NO: 129); sfu-t2 (SEQ ID
NO:130); tnfr2 (SEQ ID NO:131); cd40 (SEQ ID NO:132);
35   osteo (SEQ ID NO:133); ngfr (SEQ ID NO:134); ox40 (SEQ
ID NO:135]; 41bb (SEQ ID NO:136).

Figure 3. PepPlot analysis (Wisconsin GCG Package,
Version 8.1) of the predicted rat OPG sequence. A.
Schematic representation of rat OPG showing hydrophobic
(up) and hydrophilic (down) amino acids. Also shown are
5   basic (up) and acidic (down) amino acids. B. Display of
amino acid residues that are beta-sheet forming (up)
and beta-sheet breaking down) as defined by Chou and
Fasman (Adv. Enz. 47, 45-147 (1948)). C. Display of
propensity measures for alpha-helix and beta-sheet
10   (Chou and Fasman, ibid). Curves above 1.00 show
propensity for alpha-helix or beta-sheet structure.
Structure may terminate in regions of protein where
curves drop below 1.00. D. Display of residues that are
alpha-forming (up) or alpha-breaking (down). E. Display
15   of portions of the protein sequence that resemble
sequences typically found at the amino end of alpha and
beta structures (Chou and Fasman, ibid). F. Display of
portions of the protein sequence that resemble
sequences typically found at the carboxyl end of alpha
20   and beta structures (Chou and Fasman, ibid). G. Display
of portions of the proteins sequence typically found in
turns (Chou and Fasman, ibid) H. Display of the helical
hydrophobic moment (Eisenberg et al. Proc. Natl. Acad.
Sci. USA 81, 140-144 (1984)) at each position in the
25   sequence. I. Display of average hydropathy based upon
Kyte and Doolittle (J. Mol. Biol. 157, 105-132 (1982))
and Goldman et al. (reviewed in Ann. Rev. Biophys.
Biophys. Chem. 15, 321-353 (1986)).

Figure 4. mRNA expression patterns for the OPG
30   cDNA in human tissues. Northern blots were probed with
a 32P-labeled rat cDNA insert (A, left two panels), or
with the human cDNA insert (B, right panel).

Figure 5. Creation of transgenic mice expressing
the OPG cDNA in hepatocytes. Northern blot expression
35   of HE-OPG transgene in mouse liver.

Figure 6. Increase in bone density in OPG
transgenic mice. Panel A-F. Control Mice. G-J, OPG
expressing mice. At necropsy, all animals were
radiographed and photographs prepared. In A-F, the
5   radiographs of the control animals and the one
transgenic non-expressor (#28) are shown. Note that the
bones have a clearly defined cortex and a lucent
central marrow cavity. In contrast, the OPG (G-J)
animals have a poorly defined cortex and increased
10   density in the marrow zone.

Figure 7. Increase in trabecular bone in OPG
transgenic mice. A-D. Representative photomicrographs
of bones from control animals. In A and B, low (4X,
10X) power images of the femurs are shown (Masson
15   Trichrome stain). Stains for tartrate resistant acid
phosphatase (TRAP) demonstrate osteoclasts (see arrows)
both resorbing cartilage (C) and trabecular bone (D).
Note the flattened appearance of osteoclasts on
trabecular bone. E-H. Representative photomicrographs
20   of bones from OPG-expressing animals. In E and F, low
(4X, 10X) power images of the femurs are shown (Masson
Trichrome stain). The clear region is the growth plate
cartilage, blue stained area is bone, and the red area
is marrow. Note that in contrast to the controls, the
25   trabecular bone has not been resorbed resulting in the
absence of the usual marrow cavity. Also, the resulting
trabeculae have a variegated appearance with blue and
clear areas. The clear areas are remnants of growth
plate cartilage that have never been remodelled. Based
30   on TRAP stains, these animals do have osteoclasts (see
arrows) at the growth plate (G), which may be reduced
in number. However, the surfaces of the trabeculae away
from the growth plate are virtually devoid of
osteoclasts (H), a finding that stands in direct
35   contrast with the control animals (see D).

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Figure 8. HE-OPG expressors do not have a defect
in monocyte-macrophage development. One cause for
osteopetrosis in mice is defective M-CSF production due
to a point mutation in the M-CSF gene. This results in
5   a marked deficit of circulating and tissue based
macrophages. The peripheral blood of OPG expressors
contained monocytes as assessed by H1E analysis. To
affirm the presence of tissue macrophages,
immnohistochemistry was performed using F480
10 antibodies, which recognize a cell surface antigen on
murine macrophages. A and C show low power (4X)
photomicrographs of the spleens from normal and CR1
overexpressors. Note that both animals have numerous
F480 positive cells. Monocyte-macrophages were also
15 present in the marrow of normal (B) and HE-OPG
overexpressors (D) (40X).

Figure 9. Structure and sequence of mouse and
human OPG cDNA clones. A, B. Mouse cDNA and protein
sequence. C, D. Human cDNA and protein sequence. The
20 predicted signal peptides are underlined, and potential
sites of N-linked glycosylation are indicated in bold.
E, F. Sequence alignment and comparison of rat, mouse
and human OPG amino acid sequences.

Figure 10. Comparison of conserved sequences in
25 extracellular domain of TNFR-I and human OPG.
PrettyPlot (Wisconsin GCG Package, Version 8.1) of the
TNFR1 and OPG alignment described in example 6. Top
line, human TNFR1 sequences encoding domains 1-4.
Bottom line, human OPG sequences encoding domains 1-4.
30 Conserved residues are highlighted by rectangular
boxes.

Figure 11. Three-dimensional representation of
human OPG. Side-view of the Molescript display of the
predicted 3-dimensional structure of human OPG residues
35 25 through 163, (wide line), co-crystallized with human

TNFβ (thin line). As a reference for orientation, the
bold arrows along the OPG polypeptide backbone are
pointing in the N-terminal to C-terminal direction. The
location of individual cysteine residue side chains are
5   inserted along the polypeptide backbone to help
demonstrate the separate cysteine-rich domains. The
TNFβ molecule is aligned as described by Banner et al.
(1993).

Figure 12. Structure of OPG cysteine-rich domains.
10 Alignment of the human (top line SEQ ID NO:136) and
mouse (bottom line) OPG amino acid sequences
highlighting the predicted domain structure of OPG. The
polypeptide is divided into two halves; the N-terminus
(A), and C-terminus (B). The N-terminal half is
15 predicted to contain four cysteine rich domains
(labeled 1-4). The predicted intrachain disulfide bonds
are indicated by bold lines, labeled "SS1", "SS2", or
"SS3". Tyrosine 28 and histidine 75 (underlined) are
predicted to form an ionic interaction. Those amino
20 acids predicted to interact with an OPG ligand are
indicated by bold dots above the appropriate residue.
The cysteine residues located in the C-terminal half of
OPG are indicated by rectangular boxes.

Figure 13. Expression and secretion of full length
25 and truncated mouse OPG-Fc fusion proteins. A. Map
indicating points of fusion to the human IgG1 Fc domain
are indicated by arrowheads. B. Silver stain of a SDS-
polyacrylamide gel of conditioned media obtained from
cells expressing either Fl.Fc (Full length OPG fused to
30 Fc at Leucine 401) or CT.Fc (Carboxy-terminal truncated
OPG fused to Fc at threonine 180) fusion protein
expression vectors. Lane 1, parent pCEP4 expression
vector cell line; Lane 2, Fl.Fc vector cell line; Lane
3, CT.Fc vector cell line. C. Western blot of
35 conditioned media obtained from Fl.Fc and CT.Fc fusion

protein expression vectors probed with anti-human IgG1
Fc domain (Pierce). Lane 1, parent pCEP4 expression
vector cell line; Lane 2, Fl.Fc vector cell line; Lane
3, CT.Fc vector cell line.

5   Figure 14. Expression of human OPG in E. coli. A.
Construction of a bacterial expression vector. The LORF
of the human OPG gene was amplified by PCR, then joined
to a oligonucleotide linker fragment (top strand is SEQ
ID NO:137; bottom strand is SEQ ID NO:127), and ligated
10 into pAMG21 vector DNA. The resulting vector is capable
of expressing OPG residues 32-401 linked to a N-
terminal methionine residue. B SDS-PAGE analysis of
uninduced and induced bacterial harboring the pAMG21-
human OPG -32-401 plasmid. Lane 1, MW standards; lane
15 2, uninduced bacteria; lane 3, 30°C induction; lane 4,
37°C induction; lane 5, whole cell lysate from 37°C
induction; lane 6, soluble fraction of whole cell
lysate; lane 7, insoluble fraction of whole cell
lysate; lane 8, purified inclusion bodies obtained from
20 whole cell lysate.

      Figure 15. Analysis of recombinant murine OPG
produced in CHO cells by SDS-PAGE and western blotting.
An equal amount of CHO conditioned media was applied to
each lane shown, and was prepared by treatment with
25 either reducing sample buffer (left lane), or non-
reducing sample buffer (right lane). After
electrophoresis, the resolved proteins were transferred
to a nylon membrane, then probed with anti-OPG
antibodies. The relative positions of the 55 kd
30 monomeric and 100 kd dimeric forms of OPG are indicated
by arrowheads.

      Figure 16. Pulse-chase analysis of recombinant
murine OPG produced in CHO cells. CHO cells were
pulse-labeled with ^{35}S-methionine/cysteine, then chased
35 for the indicated time. Metabolically labeled cultures
were separated into both conditioned media and cells,

and detergent extracts were prepared from each,
clarified, then immunoprecipitated with anti-OPG
antibodies. The immunoprecipitates were the resolved by
SDS-PAGE, and exposed to film. Top left and right
5 panels; samples analyzed under non-reducing conditions.
Lower left and right panels; samples analyzed under
reducing conditions. Top and bottom left panels; Cell
extracts. Top and bottom right panels; Conditioned
media extracts. The relative mobility of the 55 kd
10 monomeric and 100 kd dimeric forms of OPG are indicated
by arrowheads.

     Figure 17. Expression of OPG in the CTLL-2 cell
line. Serum-free conditioned media from CTLL-2 cells
and CHO-mu OPG [1-401] transfected cells was prepared,
15 concentrated, then analyzed by non-reducing SDS-PAGE
and western blotting. Left lane; CTLL-2 conditioned
media. Right lane; CHO-muOPG conditioned media. The
relative mobility of the 55 kd monomeric and 100 kd
dimeric forms of OPG are indicated by arrowheads.

20   Figure 18. Detection of OPG expression in serum
samples and liver extracts obtained from control and
OPG transgenic mice. Transgenic mice were constructed
as described in Example 4. OPG expression was
visualized after SDS-PAGE followed by Western blotting
25 using anti-OPG antibodies.

     Figure 19. Effects of huOPG [22-401]-Fc fusion
protein on osteoclast formation _in_ _vitro_. The
osteoclast forming assay was performed as described in
Example 11A in the absence (control) or presence of the
30 indicated amounts of huOPG [22-401]-Fc fusion.
Osteoclast formation was visualized by histochemical
staining for tartrate acid phosphatase (TRAP).). A.
OPG added to 100 ng/ml. D. OPG added to 0.1 ng/ml. E.
OPG added to 0.01 ng/ml. F. OPG added to 0.001 ng/ml.
35 G. Control. No OPG added.

Figure 20. Decrease in osteoclast culture TRAP activity with increasing amounts of OPG. Indicated concentrations of huOPG [22-401]-Fc fusion protein were added to osteoclast forming assay and TRAP activity

5   quantitated as described in Example 11A.

Figure 21. Effect of OPG on a terminal stage of osteoclast differentiation. huOPG [22-401]-Fc fusion was added to the osteoclast forming assay during the intermediate stage of osteoclast maturation (days 5-6;

10 OPG-CTL) or during the terminal stage of osteoclast maturation (days 7-15; CTL-OPG). TRAP activity was quantitated and compared with the activity observed in the absence of OPG (CTL-CTL) in the presence of OPG throughout (OPG-OPG).

15 Figure 22. Effects of IL-1β, IL-1α and OPG on blood ionized calcium in mice. Levels of blood ionized calcium were monitored after injection of IL-1β alone, IL-1α alone, IL-1β plus muOPG [22-401]-Fc, IL-1α plus MuOPG [22-401]-Fc, and muOPG [22-401]-Fc alone. Control

20 mice received injections of phosphate buffered saline (PBS) only. IL-1β experiment shown in A; IL-1α experiment shown in B.

Figure 23. Effects of OPG on calvarial osteoclasts in control and IL-1-treated mice. Histological methods

25 for analyzing mice calvarial bone samples are described in Example 11B. Arrows indicate osteoclasts present in day 2-treated mice. Calvarial samples of mice receiving four PBS injections daily (A), one injection of IL-1 and three injections of PBS daily (B), one injection of

30 PBS and three injections of OPG daily (C), one injection of IL-1 and three injections of OPG daily.

Figure 24. Radiographic analysis of bone accumulation in marrow cavity of normal mice. Mice were injected subcutaneously with saline (A) or muOPG [22-

401]-Fc fusion (5mg/kg/d) for 14 days (B) and bone
density determined as described in Example 11C.

Figure 25. Histomorphometric analysis of bone
accumulation in marrow cavity of normal mice. Injection
5   experiments and bone histology performed as described
in Example 11C.

Figure 26. Histology analysis of bone accumulation
in marrow cavity of normal mice. Injection experiments
and bone histology performed as described in Example
10 11C. A. Saline injection B. Injection of muOPG [22-
401]-Fc fusion.

Figure 27. Activity of OPG administered to
ovariectomized rats. In this two week experiment the
trend to reduced bone density appears to be blocked by
15 OPG or other anti-resorptive therapies. DEXA
measurements were taken at time of ovariectomy and at
week 1 and week 2 of treatment. The results are
expressed as % change from the initial bone density
(Mean +/- SEM).

20   Figure 28. Bone density in the femoral metaphysis,
measured by histomorphometric methods, tends to be
lower in ovariectomized rats (OVX) than sham operated
animals (SHAM) 17 days following ovariectomy. This
effect was blocked by OPG-Fc, with OPG-Fc treated
25 ovariectomized rats (OVX+OPG) having significantly
higher bone density than vehicle treated ovariectomized
rats (OVX). (Mean +/- SEM).

Figure 29A through 29G. Sequence of OPG-Fc. DNA
and encoded protein sequences are shown. Restriction
30 sites for various nucleases are noted above the DNA
sequence.

Figures 30A through 30D. Effects of OPG-Fc during
the course of adjuvant arthritis I male Lewis rats.
Paws from rats with adjuvant arthritis induced by 0.5mg
35 mycobacteria in oil were analyzed by DEXA for bone
mineral density (BMD). Evaluation of BMD, a 29mm X 25mm

box was centered at the calcaneus (expt AdA-14 2/99,
Amgen nb#22957 p47-49). * compared to normal, #
compared to vehicle P<0.05 Mann-Whitney U test.

5   Figures 31A and 31B. Combination treatment with
OPG-Fc and sTNF-RI on Adjuvant Arthritis in Male Lewis
Rats. Area under the curve (AUC) for measurement of paw
swelling and BMD were measured as described above for
Figure 33 and in the examples hereinafter.

Detailed Description of the Invention

OPG proteins

    The term "OPG protein" refers collectively to the
novel member of the tumor necrosis factor receptor
5   family described hereinafter, variants and truncations
thereof that maintain OPG's activity in increasing bone
density, and antibodies to OPG ligand that maintain
OPG's activity in increasing bone density. An exemplary
assay for measuring such activity is shown in figure 6
10 and the accompanying text. Exemplary OPG proteins are
polypeptides comprising the consensus of the rat, mouse
and human sequences (figure 9C), OPG-Fc fusions
(figures 13, 29), or the rat, mouse or human OPG
sequences (figures 2, 9).
15   OPG was identified as follows. A novel member of
the tumor necrosis factor receptor (TNFR) superfamily
was identified as an expressed sequence tag (EST)
isolated from a fetal rat intestinal cDNA library . The
structures of the full-length rat cDNA clones and the
20 corresponding mouse and human cDNA clones were
determined as described in Examples 1 and 6. The rat,
mouse and human genes are shown in Figures 2B-2C (SEQ
ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D (SEQ ID
NO:124), respectively. All three sequences showed
25 strong similarity to the extracellular domains of TNFR
family members. None of the full-length cDNA clones
isolated encoded transmembrane and cytoplasmic domains
that would be expected for membrane-bound receptors,
suggesting that these cDNAs encode soluble, secreted
30 proteins rather than cell surface receptors. A portion
of the human gene spanning nucleotides 1200-1353 shown
in Figure 9D was deposited in the Genebank database on
November 22, 1995 under accession no. 17188769.

    The tissue distribution of the rat and human mRNA
35 was determined as described in Example 2. In rat, mRNA
expression was detected in kidney, liver, placenta and

heart with the highest expression in the kidney.
Expression in skeletal muscle and pancreas was also
detected. In humans, expression was detected in the
same tissues along with lymph node, thymus, spleen and
5   appendix.
      The rat cDNA was expressed in transgenic mice
(Example 3) using the liver-specific ApoE promoter
expression system. Analysis of expressors showed a
marked increase in bone density, particularly in long
10   bones (femurs), vertebrae and flat bones (pelvis).
Histological analysis of stained sections of bone
showed severe osteopetrosis (see Example 4) indicating
a marked imbalance between bone formation and
resorption which has led to a marked accumulation of
15   bone and cartilage. A decrease in the number of
trabecular osteoclasts in the bones of OPG expressor
animals indicate that a significant portion of the
activity of the TNFR-related protein may be to prevent
bone resorption, a process mediated by osteoclasts. In
20   view of the activity in transgenic expressors, the
TNFR-related proteins described herein are termed OPGs.
      Using the rat cDNA sequence, mouse and human cDNA
clones were isolated (Example 5). Expression of mouse
OPG in 293 cells and human OPG in E. coli is described
25   in Examples 7 and 8. Mouse OPG was produced as an Fc
fusion which was purified by Protein A affinity
chromatography. Also described in Example 7 is the
expression of full-length and truncated human and mouse
OPG polypeptides in CHO and 293 cells either as fusion
30   polypeptides to the Fc region of human IgG1 or as
unfused polypeptides. The expression of full-length and
truncated human and mouse OPGs in E. coli either as Fc
fusion polypeptides or as unfused polypeptides is
described in Example 8. Purification of recombinantly
35   produced mammalian and bacterial OPG is described in
Example 10.

The biological activity of OPG was determined
using an in vitro osteoclast maturation assay, an in
vivo model of interleukin-1 (IL-1) induced
hypercalcemia, and injection studies of bone density in
5   normal mice (see Example 11). The following OPG
recombinant proteins produced in CHO or 293 cells
demonstrated activity in the in E. coli osteoclast
maturation assay: muOPG [22-185]-Fc, muOPG [22-194]-Fc,
muOPG [22-401]Fc, muOPG [22-401], huOPG [22-201]-Fc,
10 huOPG [22-401]-Fc. muOPG [22-180]-Fc produced in CHO
cells and huOPG met[32-401] produced in E. coli did not
demonstrate activity in the in vitro assay.
     OPG from several sources was produced as a dimer
and to some extent as a higher multimer. Rat OPG [22-
15 401] produced in transgenic mice, muOPG [22-401] and
huOPG [22-401] produced as a recombinant polypeptide in
CHO cells, and OPG expressed as a naturally occurring
product from a cytotoxic T cell line were predominantly
dimers and trimers when analyzed on nonreducing SDS
20 gels (see Example 9). Truncated OPG polypeptides having
deletions in the region of amino acids 186-401 (e.g.,
OPG [1-185] and OPG [1-194]) were predominantly
monomeric suggesting that the region 186-401 may be
involved in self-association of OPG polypeptides.
25 However, huOPG met[32-401] produced in E. coli was
largely monomeric.
     OPG may be important in regulating bone
resorption. The protein appears to act as a soluble
receptor of the TNF family and may prevent a receptor-
30 ligand interaction involved in the osteolytic pathway.
One aspect of the regulation appears to be a reduction
in the number of osteoclasts.
     OPG proteins encompassed by the invention include
rat [1-401], rat [22-180], rat [22-401], rat [22-401]-
35 Fc fusion, rat [1-180]-Fc fusion, mouse [1-401], mouse
[1-180], mouse [22-401], human [1-401], mouse [22-180],

human [22-401], human [22-180], human [1-180], human
[22-180]-Fc fusion and human met-32-401. Amino acid
numbering is as shown in SEQ ID NO:121 (rat), SEQ ID
NO:123 (mouse) and SEQ ID NO:125 (human). Also

5   encompassed are polypeptide derivatives having
deletions or carboxy-terminal truncations of part or
all of amino acids residues 180-401 of OPG; one or more
amino acid changes in residues 180-401; deletion of
part or all of a cysteine-rich domain of OPG, in

10   particular deletion of the distal (carboxy-terminal)
cysteine-rich domain; and one or more amino acid
changes in a cysteine-rich domain, in particular in the
distal (carboxy-terminal) cysteine-rich domain. In one
embodiment, OPG has from 1 to about 216 amino acids

15   deleted from the carboxy terminus. In another
embodiment, OPG has from 1 to about 10 amino acids
deleted from the mature amino terminus (wherein the
mature amino terminus is at residue 22) and,
optionally, has from 1 to about 216 amino acids deleted

20   from the carboxy terminus.

     Additional OPG proteins encompassed by the
invention include the following: human [22-180]-Fc
fusion, human [22-201]-Fc fusion, human [22-401]-Fc
fusion, mouse [22-185]-Fc fusion, mouse [22-194]-Fc

25   fusion. These polypeptides are produced in mammalian
host cells, such as CHO or 293 cells, Additional OPG
polypeptides encompassed by the invention which are
expressed in procaryotic host cells include the
following: human met[22-401], Fc-human met[22-401]

30   fusion (Fc region is fused at the amino terminus of the
full-length OPG coding sequence as described in Example
8), human met[22-401]-Fc fusion (Fc region fused to the
full-lengh OPG sequence), Fc-mouse met[22-401] fusion,
mouse met[22-401]-Fc fusion, human met[27-401], human

35   met[22-185], human met[22-189], human met[22-194],
human met[22-194] (P25A), human met [22-194] (P26A),

human met[27-185], human met[27-189], human met[27-
194], human met-arg-gly-ser-(his)6 [22-401], human met-
lys [22-401], human met-(lys)3-[22-401], human met[22-
401]-Fc (P25A), human met[22-401](P25A), human
5   met[22-401](P26A), human met[22-401] (P26D), mouse
met[22-401], mouse met[27-401], mouse met[32-401],
mouse met[27-180], mouse met[22-189], mouse met[22-
194], mouse met[27-189], mouse met[27-194], mouse
met-lys[22-401], mouse HEK[22-401](A45T), mouse met-
10   lys-(his)7[22-401], mouse met-lys[22-401]-(his)7 and
mouse met[27-401] (P33E, G36S, A45P). It is understood
that the above OPG polypeptides produced in procaryotic
host cells have an amino-terminal methionine residue,
if such a residue is not indicated. In specific
15   examples, OPG-Fc fusion were produced using a 227 amino
acid region of human IgG1-γ1 was used having the
sequence as shown in Ellison et al. (1982) Nuc. Acids
Res. 10: 4071-9. However, variants of the Fc region of
human IgG may also be used.
20   Analysis of the biological activity of carboxy-
terminal OPG truncations fused to the human IgG1 Fc
region indicates a portion of OPG of about 164 amino
acids which is required for activity. This region
encompasses amino acids 22-185, preferably those in
25   Figure 9C-9D (SEQ ID NO:125), and comprises four
cysteine-rich domains characteristic of the cysteine-
rich domains of TNFR extraceullular domains. Proteins
comprising this 164 amino acid sequence are within the
meaning of "OPG protein" in this invention.
30   OPG proteins of the invention also may be isolated
and purified from other polypeptides present in
tissues, cell lines and transformed host cells
expressing OPG, or purified from components in cell
cultures containing the secreted protein. In one
35   embodiment, the polypeptide is free from association

with other human proteins, such as the expression
product of a bacterial host cell.

   A method for the purification of OPG from natural
sources and from transfected host cells is also
5   included. The purification process may employ one or
more standard protein purification steps in an
appropriate order to obtain purified protein. The
chromatography steps can include ion exchange, gel
filtration, hydrophobic interaction, reverse phase,
10   chromatofocusing, affinity chromatography employing an
anti-OPG antibody or biotin-streptavidin affinity
complex and the like.

IL-1 inhibitors

   One of the most potent inflammatory cytokines yet
15   discovered is interleukin-1 (IL-1). IL-1 is thought to
be a key mediator in many diseases and medical
conditions. It is manufactured (though not exclusively)
by cells of the macrophage/monocyte lineage and may be
produced in two forms: IL-1 alpha (IL-1α) and IL-1 beta
20   (IL-1β).

   A disease or medical condition is considered to be
an "interleukin-1 mediated disease" if the spontaneous
or experimental disease or medical condition is
associated with elevated levels of IL-1 in bodily
25   fluids or tissue or if cells or tissues taken from the
body produce elevated levels of IL-1 in culture. In
many cases, such interleukin-1 mediated diseases are
also recognized by the following additional two
conditions: (1) pathological findings associated with
30   the disease or medical condition can be mimicked
experimentally in animals by administration of IL-1 or
upregulation of expression of IL-1; and (2) a pathology
induced in experimental animal models of the disease or
medical condition can be inhibited or abolished by
35   treatment with agents that inhibit the action of IL-1.
In most interleukin-1 mediated diseases at least two of

the three conditions are met, and in many interleukin-1 mediated diseases all three conditions are met.

A non-exclusive list of acute and chronic interleukin-1 (IL-1)-mediated diseases includes but is
5   not limited to the following:

acute pancreatitis;

ALS;

Alzheimer's disease;

cachexia/anorexia, including AIDS-induced
10   cachexia;

asthma and other pulmonary diseases; atherosclerosis;

autoimmune vasculitis;

chronic fatigue syndrome;
15   Clostridium associated illnesses, including Clostridium-associated diarrhea;

coronary conditions and indications, including congestive heart failure, coronary restenosis, myocardial infarction, myocardial
20   dysfunction (e.g., related to sepsis), and coronary artery bypass graft;

cancer, such as multiple myeloma and myelogenous (e.g., AML and CML) and other leukemias, as well as tumor metastasis;
25   diabetes (e.g., insulin diabetes);

endometriosis;

fever;

fibromyalgia;

glomerulonephritis;
30   graft versus host disease/transplant rejection;

hemohorragic shock;

hyperalgesia;

inflammatory bowel disease;

inflammatory conditions of a joint, including
osteoarthritis, psoriatic arthritis and rheumatoid
arthritis;

inflammatory eye disease, as may be
5   associated with, for example, corneal transplant;

ischemia, including cerebral ischemia (e.g.,
brain injury as a result of trauma, epilepsy,
hemorrhage or stroke, each of which may lead to
neurodegeneration);
10   Kawasaki's disease;

learning impairment;

lung diseases (e.g., ARDS);

multiple sclerosis;

myopathies (e.g., muscle protein metabolism,
15   esp. in sepsis);

neurotoxicity (e.g., as induced by HIV);

osteoporosis;

pain, including cancer-related pain;

Parkinson's disease;
20   periodontal disease;

pre-term labor;

psoriasis;

reperfusion injury;

septic shock;
25   side effects from radiation therapy;

temporal mandibular joint disease;

sleep disturbance;

uveitis;

or an inflammatory condition resulting from
30   strain, sprain, cartilage damage, trauma, orthopedic
surgery, infection or other disease processes.

Interleukin-1 inhibitors may be from any protein
capable of specifically preventing activation of
cellular receptors to IL-1, which may result from any
35   number of mechanisms. Such mechanisms include
downregulating IL-1 production, binding free IL-1,

interfering with IL-1 binding to its receptor,
interfering with formation of the IL-1 receptor complex
(i.e., association of IL-1 receptor with IL-1 receptor
accessory protein), or interfering with modulation of
5   IL-1 signaling after binding to its receptor. Classes
of interleukin-1 inhibitors include:
        interleukin-1 receptor antagonists such as IL-1ra,
as described below;
        anti-IL-1 receptor monoclonal antibodies (e.g., EP
10   623674), the disclosure of which is hereby incorporated
by reference;
        IL-1 binding proteins such as soluble IL-1
receptors (e.g., U. S. Pat. No. 5,492,888, U. S. Pat.
No. 5,488,032, and U. S. Pat. No. 5,464,937, U. S. Pat.
15   No. 5,319,071, and U.S. Pat. No. 5,180,812, the
disclosures of which are hereby incorporated by
reference);
        anti-IL-1 monoclonal antibodies (e.g., WO 9501997,
WO 9402627, WO 9006371, U.S.Pat. No. 4,935,343, EP
20   364778, EP 267611 and EP 220063, the disclosures of
which are hereby incorporated by reference);
        IL-1 receptor accessory proteins and antibodies
thereto (e.g., WO 96/23067 and WO 99/37773, the
disclosure of which is hereby incorporated by
25   reference);
        inhibitors of interleukin-1 beta converting enzyme
(ICE) or caspase I (e.g., WO 99/46248, WO 99/47545, and
WO 99/47154, the disclosures of which are hereby
incorporated by reference), which can be used to
30   inhibit IL-1 beta production and secretion;
        interleukin-1beta protease inhibitors;
        and other compounds and proteins which block *in
vivo* synthesis or extracellular release of IL-1.
        Exemplary IL-1 inhibitors are disclosed in the
35   following references:

US Pat. Nos. 5,747,444; 5,359,032; 5,608,035;
5,843,905; 5,359,032; 5,866,576; 5,869,660; 5,869,315;
5,872,095; 5,955,480; 5,965,564;

International (WO) patent applications 98/21957,
5 96/09323, 91/17184, 96/40907, 98/32733, 98/42325,
98/44940, 98/47892, 98/56377, 99/03837, 99/06426,
99/06042, 91/17249, 98/32733, 98/17661, 97/08174,
95/34326, 99/36426, 99/36415.

European (EP) patent applications 534978 and
10 894795.

French patent application FR 2762514.
The disclosures of all of the aforementioned references
are hereby incorporated by reference.

For purposes of the present invention, IL-1ra and
15 variants and derivatives thereof as discussed
hereinafter are collectively termed "IL-1ra
protein(s)". The molecules described in the above
references and the variants and derivatives thereof
discussed hereinafter are collectively termed "IL-1
20 inhibitors."

Interleukin-1 receptor antagonist (IL-1ra) is a
human protein that acts as a natural inhibitor of
interleukin-1 and which is a member of the IL-1 family
member which includes IL-1α and IL-1β. Preferred
25 receptor antagonists (including IL-1ra and variants and
derivatives thereof), as well as methods of making and
using thereof, are described in U.S. Patent No.
5,075,222; WO 91/08285; WO 91/17184; AU 9173636;
WO 92/16221; WO93/21946; WO 94/06457; WO 94/21275;
30 FR 2706772; WO 94/21235; DE 4219626, WO 94/20517; WO
96/22793;WO 97/28828; and WO 99/36541, the disclosures
of which are incorporated herein by reference. The
proteins include glycosylated as well as non-
glycosylated IL-1 receptor antagonists.

Specifically, three useful forms of IL-1ra and
variants thereof are disclosed and described in the
5,075,222 patent. The first of these, called "IL-1i" in
the '222 patent, is characterized as a 22-23 kD
5   molecule on SDS-PAGE with an approximate isoelectric
point of 4.8, eluting from a Mono Q FPLC column at
around 52 mM NaCl in Tris buffer, pH 7.6. The second,
IL-1raβ, is characterized as a 22-23 kD protein,
eluting from a Mono Q column at 48 mM NaCl. Both IL-
10 1raα and IL-1raβ are glycosylated. The third, IL-1rax,
is characterized as a 20 kD protein, eluting from a
Mono Q column at 48 mM NaCl, and is non-glycosylated.
5,075,222 patent also discloses methods for isolating
the genes responsible for coding the inhibitors,
15 cloning the gene in suitable vectors and cell types,
and expressing the gene to produce the inhibitors.

Those skilled in the art understand that many
combinations of deletions, insertions and substitutions
(individually or collectively "variant(s)") can be made
20 within the amino acid sequences of IL-1ra, provided
that the resulting molecule is biologically active
(e.g., possesses the ability to inhibit IL-1). See
"Variants of Proteins" hereinafter.

TNF-α inhibitors

25 Many diseases and medical conditions are mediated
by TNF and are usually categorized as inflammatory
conditions. A "TNF-mediated disease" is a spontaneous
or experimental disease or medical condition is
associated with elevated levels of TNF in bodily fluids
30 or tissue or if cells or tissues taken from the body
produce elevated levels of TNF in culture. In many
cases, such TNF-mediated diseases may also be
recognized by (1) pathological findings associated with
the disease or medical condition can be mimicked
35 experimentally in animals by the administration or

upregulation of expression of TNF or (2) a pathology
induced in experimental animal models of the disease or
medical condition can be inhibited or abolished by
treatment with agents that inhibit the action of TNF. A

5   non-exclusive list of acute and chronic TNF-mediated
diseases includes but is not limited to the following:

     cachexia/anorexia;

     cancer (e.g., leukemias);

     chronic fatigue syndrome;

10   coronary conditions and indications, including
congestive heart failure, coronary restenosis,
myocardial infarction, myocardial dysfunction (e.g.,
related to sepsis), and coronary artery bypass graft;

     depression;

15   diabetes, including juvenile onset Type 1,
diabetes mellitus, and insulin resistance (e.g., as
associated with obesity);

     endometriosis, endometritis, and related
conditions;

20   fibromyalgia or analgesia;

     graft versus host rejection;

     hyperalgesia;

     inflammatory bowel diseases, including Crohn's
disease and <u>Clostridium difficile</u>-associated diarrhea;

25   ischemia, including cerebral ischemia (brain
injury as a result of trauma, epilepsy, hemorrhage or
stroke, each of which may lead to neurodegeneration);

     lung diseases (e.g., adult respiratory distress
syndrome, asthma, and pulmonary fibrosis);

30   multiple sclerosis;

     neuroinflammatory diseases;

     ocular diseases and conditions, including corneal
transplant, ocular degeneration and uveitis;

     pain, including cancer-related pain;

35   pancreatitis;

     periodontal diseases;

Pityriasis rubra pilaris (PRP);

prostatitis (bacterial or non-bacterial) and related conditions;

psoriasis and related conditions;

5   pulmonary fibrosis;

reperfusion injury;

rheumatic diseases, including rheumatoid arthritis, osteoarthritis, juvenile (rheumatoid) arthritis, seronegative polyarthritis, ankylosing

10   spondylitis, Reiter's syndrome and reactive arthritis, Still's disease, psoriatic arthritis, enteropathic arthritis, polymyositis, dermatomyositis, scleroderma, systemic sclerosis, vasculitis (e.g., Kawasaki's disease), cerebral vasculitis, Lyme disease,

15   staphylococcal-induced ("septic") arthritis, Sjögren's syndrome, rheumatic fever, polychondritis and polymyalgia rheumatica and giant cell arteritis);

septic shock;

side effects from radiation therapy;

20   systemic lupus erythematosus (SLE);

temporal mandibular joint disease;

thyroiditis;

tissue transplantation or an inflammatory condition resulting from strain, sprain, cartilage

25   damage, trauma, orthopedic surgery, infection (e.g., HIV, Clostridium difficile and related species) or other disease process.

TNF-α inhibitors may act by downregulating or inhibiting TNF production, binding free TNF,

30   interfering with TNF binding to its receptor, or interfering with modulation of TNF signaling after binding to its receptor. The term "TNF-α inhibitor" thus includes solubilized TNF receptors, antibodies to TNF, antibodies to TNF receptor, inhibitors of TNF-α

converting enzyme (TACE), and other molecules that affect TNF activity.

TNF-α inhibitors of various kinds are disclosed in the art, including the following references:

5   European patent applications 308 378; 422 339; 393 438; 398 327; 412 486; 418 014, 417 563, 433 900; 464 533;512 528; 526 905;568 928; EP 607 776 (use of leflunomide for inhibition of TNF-α); 663 210; 542 795; 818 439; 664 128; 542 795; 741 707; 874 819 ; 882 714;

10   880 970; 648 783; 731 791; 895 988; 550 376; 882 714; 853 083; 550 376; 943 616; 939 121; 614 984 ; 853 083

U.S. Patent Nos. 5,136,021; 5,929,117; 5,948,638; 5,807,862; 5,695,953; 5,834,435; 5,817,822; 5830742; 5,834,435; 5,851,556; 5,853,977; 5,359,037; 5,512,544;

15   5,695,953; 5,811,261; 5,633,145; 5,863,926; 5,866,616; 5,641,673; 5,869,677; 5,869,511; 5,872,146; 5,854,003; 5,856,161; 5,877,222; 5,877,200; 5,877,151; 5,886,010; 5,869,660; 5,859,207; 5,891,883; 5,877,180; 5,955,480; 5,955,476; 5,955,435; 5,994,351; 5,990,119; 5,952,320;

20   5,962,481;

International(WO)patent applications 90/13575, 91/03553, 92/01002, 92/13095, 92/16221, 93/07863, 93/21946, 93/19777, 95/34326, 96/28546, 98/27298, 98/30541, 96/38150, 96/38150, 97/18207, 97/15561,

25   97/12902, 96/25861, 96/12735, 96/11209, 98/39326, 98/39316, 98/38859, 98/39315, 98/42659, 98/39329, 98/43959, 98/45268, 98/47863, 96/33172, 96/20926, 97/37974, 97/37973, 97/47599, 96/35711, 98/51665, 98/43946, 95/04045, 98/56377, 97/12244, 99/00364,

30   99/00363, 98/57936, 99/01449, 99/01139, 98/56788, 98/56756, 98/53842, 98/52948, 98/52937, 99/02510, 97/43250, 99/06410, 99/06042, 99/09022, 99/08688, 99/07679, 99/09965, 99/07704, 99/06041, 99/37818, 99/37625, 97/11668, 99/50238, 99/47672, 99/48491;

Japanese (JP) patent applications 10147531,
10231285, 10259140, and 10130149, 10316570, 11001481,
and 127,800/1991;

German (DE) application 19731521;

5   British (GB) applications 2 218 101, 2 326 881, 2
246 569.

The disclosures of all of the aforementioned
references are hereby incorporated by reference.

For purposes of this invention, the molecules
10   disclosed in these references and the sTNFRs and
variants and derivatives of the sTNFRs and the
molecules disclosed in the references (see below) are
collectively termed "TNF-α inhibitors."

For example, EP 393 438 and EP 422 339 teach the
15   amino acid and nucleic acid sequences of a soluble TNF
receptor type I (also known as sTNFR-I or 30kDa TNF
inhibitor) and a soluble TNF receptor type II (also
known as sTNFR-II or 40kDa TNF inhibitor), collectively
termed "sTNFRs", as well as modified forms thereof
20   (e.g., fragments, functional derivatives and variants).
EP 393 438 and EP 422 339 also disclose methods for
isolating the genes responsible for coding the
inhibitors, cloning the gene in suitable vectors and
cell types, and expressing the gene to produce the
25   inhibitors.

sTNFR-I and sTNFR-II are members of the nerve
growth factor/TNF receptor superfamily of receptors
which includes the nerve growth factor receptor (NGF),
the B cell antigen CD40, 4-1BB, the rat T-cell antigen
30   MRC OX40, the fas antigen, and the CD27 and CD30
antigens (Smith et al. (1990), Science, 248:1019-1023).
The most conserved feature amongst this group of cell
surface receptors is the cysteine-rich extracellular
ligand binding domain, which can be divided into four
35   repeating motifs of about forty amino acids and which

contains 4-6 cysteine residues at positions which are
well conserved (Smith et al. (1990), supra).

　　　EP 393 438 teaches a 40kDa TNF inhibitor Δ51 and a
40kDa TNF inhibitor Δ53, which are truncated versions
of the full-length recombinant 40kDa TNF inhibitor
protein wherein 51 or 53 amino acid residues,
respectively, at the carboxyl terminus of the mature
protein are removed.

　　　PCT Application No. PCT/US97/12244 teaches
truncated forms of sTNFR-I and sTNFR-II which do not
contain the fourth domain (amino acid residues Thr^{127}–
Asn^{161} of sTNFR-I and amino acid residues Pro^{141}–Thr^{179}
of sTNFR-II); a portion of the third domain (amino acid
residues Asn^{111}–Cys^{126} of sTNFR-I and amino acid
residues Pro^{123}–Lys^{140} of sTNFR-II); and, optionally,
which do not contain a portion of the first domain
(amino acid residues Asp^{1}–Cys^{19} of sTNFR-I and amino
acid residues Leu^{1}–Cys^{32} of sTNFR-II). The truncated
sTNFRs of the present invention include the proteins
represented by the formula R_1-[Cys^{19}-Cys^{103}]-R_2 and R_4-
[Cys^{32}-Cys^{115}]-R_5. These proteins are truncated forms of
sTNFR-I and sTNFR-II, respectively.

　　　By "R_1-[Cys^{19}-Cys^{103}]-R_2" is meant one or more
proteins wherein [Cys^{19}-Cys^{103}] represents residues 19
through 103 of sTNFR-I, the amino acid residue
numbering scheme of which is provided in Figure 1 to
facilitate the comparison; wherein R_1 represents a
methionylated or nonmethionylated amine group of Cys^{19}
or of amino-terminus amino acid residue(s) selected
from any one of Cys^{18} to Asp^{1} and wherein R_2 represents
a carboxy group of Cys^{103} or of carboxy-terminal amino
acid residues selected from any one of Phe^{104} to Leu^{110}.

　　　Exemplary truncated sTNFR-I of the present
invention include the following molecules (collectively

30

termed 2.6D sTNFR-I): NH_2-[Asp^1-Cys^{105}]-COOH (also
referred to as sTNFR-I 2.6D/C105); NH_2-[Asp^1-Leu^{108}]-
COOH (also referred to as sTNFR-I 2.6D/C106); NH_2-
[Asp^1-Asn^{105}]-COOH (also referred to as sTNFR-I

5   2.6D/N105); NH_2-[Tyr^9-Leu^{108}]-COOH (also referred to as
sTNFR-I 2.3D/d8); NH_2-[Cys^{19}-Leu^{108}]-COOH (also referred
to as sTNFR-I 2.3D/d18); and NH_2-[Ser^{16}-Leu^{108}]-COOH
(also referred to as sTNFR-I 2.3D/d15), either
methionylated or nonmethionylated, and variants and

10   derivatives thereof.

By "R_3-[Cys^{32}-Cys^{115}]-R_4" is meant one or more
proteins wherein [Cys^{32}-Cys^{115}] represents residues
Cys^{32} through Cys^{115} of sTNFR-II, the amino acid residue
numbering scheme of which is provided in Figure 2 to

15   facilitate the comparison; wherein R_3 represents a
methionylated or nonmethionylated amine group of Cys^{32}
or of amino-terminus amino acid residue(s) selected
from any one of Cys^{31} to Leu^1 and wherein R_4 represents
a carboxy group of Cys^{115} or of carboxy-terminal amino

20   acid residue(s) selected from any one of Ala^{116} to
Arg^{122}.

Serine Protease Inhibitors

Endogenous proteolytic enzymes degrade invading organisms, antigen-antibody complexes, and certain tissue proteins that are no longer necessary or useful.

5 Infective agents may introduce additional proteolytic enzymes into the organism. Protease inhibitors regulate both endogenous and invading proteolytic enzymes.

A large number of naturally occurring protease inhibitors serve to control the endogenous proteases by

10 limiting their reactions locally and temporally. In addition, the protease inhibitors may inhibit proteases introduced into the body by infective agents. Tissues that are particularly prone to proteolytic attack and infection, e.g. those of the respiratory tract, are

15 rich in protease inhibitors.

Protease inhibitors comprise approximately 10% of the human plasma proteins. At least eight inhibitors have been isolated from this source and characterized in the literature. These include alpha 2-macroglobulin

20 (alpha 2M), alpha 1-protease inhibitor (alpha 1PI), alpha 1-antichymotrypsin (alpha 1Achy), alpha 1-anticollagenase (alpha 1AC), and inter-alpha-trypsin inhibitor (I-alpha-I).

A disturbance of the protease/protease inhibitor

25 balance can lead to protease-mediated tissue destruction, including emphysema, arthritis, glomerulonephritis, periodontitis, muscular dystrophy, tumor invasion and various other pathological conditions. In certain situations, e.g. severe

30 pathological processes such as sepsis or acute leukemia, the amount of free proteolytic enzymes present increases due to the release of enzyme from the secretory cells. In addition, or separately in other situations, a diminished regulating inhibitor capacity

of the organism may also cause alterations in the
protease/protease inhibitor balance. An example of such
a diminished regulating inhibitor capacity is an alpha
1-protease inhibitor deficiency, which is highly
5   correlated with the development of pulmonary emphysema.

     In organisms where such aberrant conditions are
present, serious damage to the organism can occur
unless measures can be taken to control the proteolytic
enzymes. Therefore, protease inhibitors have been
10   sought which are capable of being administered to an
organism to control the proteolytic enzymes.

     One protease that is of particular pharmacological
interest is leukocyte elastase. Leukocyte elastase,
when released extracellularly, degrades connective
15   tissue and other valuable proteins. While it is
necessary for a normally functioning organism to
degrade a certain amount of connective tissue and other
proteins, the presence of an excessive amount of
leukocyte elastase has been associated with various
20   pathological states, such as emphysema and rheumatoid
arthritis. To counteract the effects of leukocyte
elastase when it is present in amounts greater than
normal, a protease inhibitor has been sought which is
specific for leukocyte elastase. Such a protease
25   inhibitor would be especially useful if it were capable
of being isolated or prepared in a purified form and in
sufficient quantities to be pharmaceutically useful

     In the past, at least two leukocyte elastase
inhibitors have been identified in the literature. One
30   protein, described in Schiessler et al., "Acid-Stable
Inhibitors of Granulocyte Neutral Proteases in Human
Mucous Secretions: Biochemistry and Possible Biological
Function", in Neutral Proteases of Human

33

Polymorphoneuclear Leucocytes, Havemann *et al.* (eds),
Urban and Schwarzenberg, Inc. (1978), was isolated from
human seminal plasma and sputum and was characterized
as being approximately 11 Kda in size with tyrosine as
5   the N-terminal amino acid. The literature reports of
this protein have only furnished a partial amino acid
sequence, but even this partial sequence indicates that
this protein varies substantially from the proteins of
the present invention. The reports of the sequence of
10 this protein, in combination with the complete amino
acid sequence data for proteins of the present
invention, indicate to the present inventors that the
product sequenced by Schiessler *et al.* may have been a
degraded protein which was not a single-polypeptide
15 chain.

     A second protein, isolated in one instance from
human plasma, has been named alpha 1-protease
inhibitor. Work on this protein has been summarized in
a review by Travis and Salvesen, *Ann. Rev. Biochem.* 52:
20 655-709 (1983). The reports of the amino acid sequence
of this protein indicate that it too differs
substantially from the proteins of the present
invention.

     Trypsin is another protease of particular interest
25 from a pharmacological standpoint. Trypsin is known to
initiate degradation of certain soft organ tissue, such
as pancreatic tissue, during a variety of acute
conditions, such as pancreatitis. A variety of efforts
have been directed toward the treatment of these
30 conditions, without marked success, through the use of
proteins which it was hoped would inhibit the action of
trypsin. Illustrative of such efforts are attempts to
use exogenous bovine trypsin inhibitors in treatment of

human pancreatitis. While such techniques have been
attempted in Europe, they have not been approved as
effective by the U.S. Food and Drug Administration.

Thus, there is a need for a protease inhibitor
5   effective in neutralizing excess trypsin in a variety
of acute and chronic conditions. As was the case with
the leukocyte elastase inhibitor discussed above, a
trypsin inhibitor would be particularly useful if it
could be isolated and prepared in a purified form and
10 in sufficient quantities to be pharmaceutically useful.

Cathepsin G is another protease present in large
quantities in leukocytes. Cathepsin G is known to be
capable of degrading in vitro a variety of valuable
proteins, including those of the complement pathway
15 Pancreatic elastase is another protease which may have
a role in pancreatitis. Thus, inhibitors for these
proteases are also of pharmaceutical value.

Leukocyte elastase, trypsin, cathepsin G and
pancreatic elastase are examples of a class of
20 proteases known as serine proteases, which have
elements of common structure and mechanism. Their
activity against different substrates and their
sensitivity to different inhibitors are believed to
result from changes in only a few amino acid residues.
25 By analogy, it is possible to conceive of a class of
serine protease inhibitors, also having common elements
of structure and mechanism, in which changes in a
relatively few amino acids will result in inhibition of
different proteases, and that at least one member of
30 this class will inhibit every serine protease of the
former class.

A particularly preferred serine protease inhibitor
is secretory leukocyte protease inhibitor (SLPI) and

fragments and analogues thereof. Also preferred are
anti-leukoprotease (ALP), mucous protease inhibitor
(MPI), human seminal plasma inhibitor-I (HUSI-I),
bronchial mucus inhibitor (BMI), cervical mucus

5   inhibitor (CUSI). These molecules are especially well-
suited for use in conditions leading to bone loss
because they are preferentially directed to the
cartilage. Exemplary serine protease inhibitors are
described in the following, each of which is hereby

10 incorporated by reference: U. S. Pat. No. 4,760,130,
issued July 26, 1988; U. S. Pat. No. 5,900,400, issued
May 4, 1999, which discloses preferred SLPI analogues;
and U. S. Pat. No. 5,633,227, issued May 27, 1997,
which discloses preferred SLPI fragments. The molecules

15 disclosed in the foregoing references as well as any
variants or analogues thereof as described hereinafter
are collectively termed "serine protease inhibitors."

IL-18 Inhibitors

IL-18 is a pro-inflammatory cytokine of somewhat

20 recent discovery. IL-18 was found to induce interferon-
γ and was originally named interferon gamma inducing
factor (IGIF). IL-1 upregulates IL-18 production, and
IL-18 induces production of a number of proinflammatory
cytokines, including IL-6 and MMP-1. Dinarello et al.

25 (1998), J. Leukocyte Biol. 63: 658-64. Caspase I is
also critical for IL-18 production. The art also
suggested that TNF-α regulates IL-18 production, and it
was found that simultaneous inhibition of TNF-α and IL-
18 protected against liver toxicity. Faggioni et al.

30 (2000), PNAS 97: 2367-72.

IL-18 acts in vivo through a receptor system
reminiscent of the IL-1 system. IL-18 interacts with a
cell surface receptor (IL-18R), which interacts with an
accessory protein (IL-18RAcP). IL-18-mediated signaling

35 proceeds upon formation of the complex of IL-18, IL-

18R, and IL-18RAcP. A natural inhibitor for IL-18 is
IL-18bp. Although it bears insignificant sequence
homology with IL-18R, IL-18bp's act as a "decoy
receptors" by binding to IL-18 molecules and preventing
5   interaction with IL-18 and subsequent IL-18-mediated
signaling.

The present invention concerns methods of
treatment using IL-18 inhibitors in combination with
the other classes of molecules described herein. Such
10   combination therapy is useful for treating inflammation
and autoimmune diseases generally, as well as IL-1
mediated diseases and TNF-mediated diseases as defined
hereinabove. In particular, combination therapy using
IL-18 inhibitors is useful for treating arthritis
15   (particularly rheumatoid arthritis), systemic lupus
erythematosus (SLE), graft versus host disease (GvHD),
hepatitis and sepsis.

A number of classes of IL-18 inhibitors are known
in the art, and all are useful in the present
20   invention. Suitable IL-18 inhibitors include antibodies
binding to IL-18; antibodies binding to IL-18R;
antibodies binding to IL-18RAcP; IL-18bp; IL-18R
fragments (e.g., a solubilized extracellular domain of
the IL-18 receptor), peptides binding to IL-18 and
25   preventing its interaction with IL-18R; peptides
binding to IL-18R and preventing its interaction with
IL-18 or with IL-18RAcP; peptides binding to IL-18RAcP
and preventing its interaction with IL-18R; and small
molecules preventing IL-18 production or interaction
30   between any of IL-18, IL-18R, and IL-18RAcP. Any of the
foregoing, with the exception of small molecules, may
be linked to half-life extending vehicles known in the
art. Such vehicles include the Fc domain, polyethylene
glycol, and dextran. These vehicles are reviewed in a
35   patent application entitled, "Modified Peptides as
Therapeutic Agents," U.S. Ser. No. 09/428,082, PCT

appl. no. WO 99/25044, which is hereby incorporated by
reference in its entirety.

Useful IL-18 inhibitors are described in the
following references, which are hereby incorporated by
5   reference: US Pat. No. 5,912,324, issued July 14, 1994;
EP 0 962 531, published Dec. 8, 1999; EP 712 931,
published Nov. 15, 1994; US Pat. No. 5,914,253, issued
July 14, 1994; WO 97/24441, published July 10, 1997; US
Pat. No. 6,060,283, issued May 9, 2000; EP 850 952,
10   published Dec. 26, 1996; EP 864 585, published Sep. 16,
1998; WO 98/41232, published Sep. 24, 1998; US Pat. No.
6,054,487, issued April 25, 2000; WO 99/09063,
published Aug 14, 1997; WO 99/22760, published Nov. 3,
1997; WO 99/37772, published Jan. 23, 1998; WO
15   99/37773, published March 20, 1998; EP 0 974 600,
published Jan. 26, 2000; WO 00/12555, published Mar. 9,
2000; Japanese patent application JP 111,399/94,
published Oct. 31, 1997; Israel patent application IL
121554 A0, published Feb. 8, 1998.
20   <u>Variants of proteins</u>

Those skilled in the art will understand that one
may make many molecules derived in sequence from the
aforementioned molecules in which amino acids have been
deleted ("deletion variants"), inserted ("addition
25   variants"), or substituted ("substitution variants").
Molecules having such substitutions, additions,
deletions, or any combination thereof are termed
individually or collectively "variant(s)"). Such
variants should, however, maintain at some level
30   (including a reduced level) the relevant activity of
the unmodified or "parent" molecule (e.g., an sTNFR
variant possesses the ability to bind TNF).
Hereinafter, "parent molecule" refers to an unmodified
molecule or a variant molecule lacking the particular
35   variation under discussion; for example, when

discussing substitution below, the parent molecule may
be a deletion variant.

Variants may be rapidly screened to assess their
physical properties. It will be appreciated that such
5   variant(s) will demonstrate similar properties to the
unmodified molecule, but not necessarily all of the
same properties and not necessarily to the same degree
as the corresponding parent molecule.

There are two principal variables in the
10   construction of amino acid sequence variant(s): the
location of the mutation site and the nature of the
mutation. In designing variant(s), the location of each
mutation site and the nature of each mutation will
depend on the biochemical characteristic(s) to be
15   modified. Each mutation site can be modified
individually or in series, e.g., by (1) deleting the
target amino acid residue, (2) inserting one or more
amino acid residues adjacent to the located site or
(3) substituting first with conservative amino acid
20   choices and, depending upon the results achieved, then
with more radical selections.

Amino acid sequence deletions generally range from
about 1 to 30 amino acid residues, preferably from
about 1 to 20 amino acid residues, more preferably from
25   about 1 to 10 amino acid residues and most preferably
from about 1 to 5 contiguous residues. Amino-terminal,
carboxy-terminal and internal intrasequence deletions
are contemplated. Deletions within the amino acid
sequences of OPG or the sTNFRs may be made, for
30   example, in regions of low homology with the sequences
of other members of the NGF/TNF receptor family. In the
case of IL-1ra, deletions may be made in regions of low
homology in the IL-1 family (which comprises IL-1 α,
IL-1 β, and IL-1ra). Deletions in areas of substantial
35   homology with other members of the family will be more

likely to significantly modify the biological activity.
Specifically, the sequence similarity among NGF/TNF
receptor family members is particularly high in the
region corresponding to the first two disulfide loops
5   of domain 1, the whole of domain 2, and the first
disulfide loop of domain 3 (Banner et al. (1993), Cell,
73:431-445). The number of total deletions and/or
consecutive deletions preferably will be selected so as
to preserve the tertiary structure in the affected
10   domain, e.g., cysteine crosslinking.
        An amino acid sequence addition may include
insertions of an amino- and/or carboxyl-terminal fusion
ranging in length from one residue to one hundred or
more residues, as well as internal intrasequence
15   insertions of single or multiple amino acid residues.
Internal additions may range generally from about 1 to
20 amino acid residues, preferably from about 1 to 10
amino acid residues, more preferably from about 1 to 5
amino acid residues, and most preferably from about 1
20   to 3 amino acid residues. Additions within the amino
acid sequences of OPG or the sTNFRs may be made in
regions of low homology with the sequences of other
members of the NGF/TNF receptor family. Additions
within the amino acid sequence of OPG or the sTNFRs in
25   areas of substantial homology with the sequences of
other members of the NGF/TNF receptor family will be
more likely to significantly modify the biological
activity. Additions preferably include amino acid
sequences derived from the sequences of the NGF/TNF
30   receptor family members.
        An amino-terminus addition is contemplated to
include the addition of a methionine (for example, as
an artifact of the direct expression in bacterial
recombinant cell culture). A further example of an
35   amino-terminal addition includes the fusion of a signal
sequence to the amino-terminus of a mature molecule in

                              40

order to facilitate its secretion from recombinant host
cells. Such signal sequences generally will be obtained
from and thus be homologous to the intended host cell
species. For prokaryotic host cells that do not
5   recognize and process the native signal sequence of the
mature molecule, the signal sequence may be substituted
by a prokaryotic signal sequence selected, for example,
from the group of the alkaline phosphatase,
penicillinase or heat-stable enterotoxin II leader
10   sequences. For expression in yeast cells the signal
sequence may be selected, for example, from the group
of the yeast invertase, alpha factor or acid
phosphatase leader sequences. For mammalian cell
expression, the native signal sequences (see, e.g., EP
15   393 438 and EP 422 339 for sTNFRs) are satisfactory,
although other mammalian signal sequences may be
suitable; for example, sequences derived from other
NGF/TNF receptor family members.

An example of an amino- or a carboxy-terminus
20   addition includes chimeric proteins comprising the
amino-terminal or carboxy-terminal fusion of the parent
molecules with all or part of the constant domain of
the heavy or light chain of human immunoglobulin
(individually or collectively, ("Fc variant(s)"). Such
25   chimeric polypeptides are preferred wherein the
immunoglobulin portion of each comprises all of the
domains except the first domain of the constant region
of the heavy chain of human immunoglobulin such as IgG
(e.g., IgG1 or IgG3), IgA, IgM or IgE. A skilled
30   artisan will appreciate that any amino acid of the
immunoglobulin portion can be deleted or substituted
with one or more amino acids, or one or more amino
acids can be added as long as the parent molecule still
maintains some level of its relevant activity and the
35   immunoglobulin portion shows one or more of its
characteristic properties.

Another group of variant(s) is amino acid
substitution variant(s). These are variant(s) wherein
at least one amino acid residue in a parent molecule is
removed and a different residue inserted in its place.

5   Substitution variant(s) include allelic variant(s)
which are characterized by naturally-occurring
nucleotide sequence changes in the species population
that may or may not result in an amino acid change. One
skilled in the art can use any information known about

10 the binding or active site of the polypeptide in the
selection of possible mutation sites.

One method for identifying amino acid residues or
regions for mutagenesis of a protein is called "alanine
scanning mutagenesis", as described by Cunningham and

15 Wells (1989), _Science_, _244_:1081-1085, the disclosure of
which is hereby incorporated by reference. In this
method, an amino acid residue or group of target
residues is identified (e.g., charged residues such as
Arg, Asp, His, Lys and Glu) and replaced by a neutral

20 or negatively-charged amino acid (most preferably
alanine or polyalanine) to affect the interaction of
the amino acids with the surrounding aqueous
environment in or outside the cell. Those
domains/residues demonstrating functional sensitivity

25 to the substitutions are then refined by introducing
additional or alternate residues at the sites of
substitution. Thus, the site for introducing an amino
acid sequence modification is predetermined. To
optimize the performance of a mutation at a given site,

30 alanine scanning or random mutagenesis may be conducted
and the variant(s) may be screened for the optimal
combination of desired activity and degree of activity.

The sites of greatest interest for substitutional
mutagenesis include sites in which particular amino

35 acid residues within a parent molecule are
substantially different from other species or other

family members in terms of side-chain bulk, charge
and/or hydrophobicity. Other sites of interest include
those in which particular residues of a parent molecule
are identical among other species or other family
5   members, as such positions are generally important for
the biological activity of a protein.

Other sites of interest include those in which
particular residues are similar or identical with
proteins with similar structure or activity to the
10 parent molecule. For sTNFR-I, for example, information
has been elucidated relevant to sTNFR-I-like molecules
(Banner et al. (1993), $supra$, and Fu et al. (1995),
$Protein$ $Engineering$, 8(12):1233-1241). Residues Tyr^9,
Thr^{39}, His^{55} in Domain 1, residues Phe^{49}, Ser^{63}, Asp^{82} in
15 Domain 2 and residues Tyr^{92} and Ser^{107} in Domain 3 have
been identified as being potentially important for the
stabilization of the structure of Domains 1, 2 and 3,
respectively. Residues Pro^{12} and His^{55} have been
identified as potentially interacting with Ser^{86}-Tyr^{87}
20 on subunit C of TNF-α. Residues Glu^{45}-Phe^{49} have been
identified as being in a loop which potentially
interacts with residues Leu^{29}-Arg^{32} of TNF-α subunit A.
Residues Gly^{48} has been identified as potentially
interacting with Asn^{19}-Pro^{20} on subunit A of TNF-α.
25 Residue His^{58}-Leu^{60} have been identified as being in an
extended strand conformation and side chain
interactions with residues Arg^{31}-Ala^{33} on subunit A of
TNF-α have been potentially identified with residue
His^{58} of sTNFR-I specifically interacting with residue
30 Arg^{31}. Residues Lys^{64}-Arg^{66} have been identified as
being in an extended strand conformation and have been
identified as having side chain and main chain

interactions with residues Ala^{145}-Glu^{146} and residue
Glu^{46} on subunit A of TNF-α. Residue Met^{69} has been
identified as potentially interacting with residue
Tyr^{115} on subunit A of TNF-α. Residues His^{94}-Phe^{101} have
5   been identified as forming a loop which interacts with
residues Thr^{72}-Leu^{75} and Asn^{137} of subunit C of TNF-α,
with residue Trp^{96} of sTNFR-I specifically interacting
with residues Ser^{71}-Thr^{72} on subunit C of TNF-α, Leu^{100}
of sTNFR-I being in close proximity with residue Asn^{137}
10 on subunit C of TNF-α and residue Gln^{102} of sTNFR-I
specifically interacting with residue Pro^{113} on subunit
A of TNF-α.

      In addition to the cysteines forming the 3 pairs
of disulfide bonds within each of the four domains of
15 the molecule, there are several other conserved
residues that contribute to the stabilization of the
tertiary fold of each domain.

      There are two main classes into which these
stabilizing residues fall. The first type contributes
20 to the shielding of the disulfide bond sulfur atoms
from solvent. An example of this residues in domain 3
is Tyr^{92}. In domain 4 Phe^{133} helps to shield the Cys^{128}-
Cys^{139} disulfide bond. All four domains have either a
Tyr or Phe at these same structurally conserved
25 locations. The second class of stabilizing residues
form hydrogen bonds within their respective domains.
Within domain 3 Asn^{123} and Ser^{107} form a hydrogen bond
and Ser^{107} forms an additional hydrogen bond with
Thr^{124}. For domain 4 these residues include Asn^{144} and
30 Ser^{141}.

      In addition there are hydrogen bonds between
domain 3 and 4 that are not seen between other domains.

These hydrogens bonds are (1) Asn[105] main-chain oxygen and Asn[137] side-chain nitrogen and (2) Ser[107] side-chain oxygen and Asn[137] main-chain nitrogen.

Another useful tool in identifying sites suitable for substitution is molecular modeling. One example of this technique is OPG. Using the homology between OPG and the extracellular ligand binding domains of TNF receptor family members, a three-dimensional model of OPG was generated based upon the known crystal structure of the extracellular domain of TNFR-I (see Example 6). This model was used to identify those residues within OPG which may be important for biological activity. Cysteine residues that are involved in maintaining the structure of the four cysteine-rich domains were identified. The following disulfide bonds were identified in the model: Domain 1: cys41 to cys54, cys44 to cys62, tyr23 and his 66 may act to stabilize the structure of this domain; Domain 2: cys65 to cys80, cys83 to cys98, cys87 to cys105; Domain 3: cys107 to cys118, cys124 to cys142; Domain 4: cys145 to cys160, cys166 to cys185. Residues were also identified which were in close proximity to TNFβ as shown in Figures 11 and 12A-12B. In this model, it is assumed that OPG binds to a corresponding ligand; TNFβ was used as a model ligand to simulate the interaction of OPG with its ligand. Based upon this modeling, the following residues in OPG may be important for ligand binding: glu34, lys43, pro66 to gln91 (in particular, pro66, his68, tyr69, tyr70, thr71, asp72, ser73, his76, ser77, asp78, glu79, leu81, tyr82, pro85, val86, lys88, glu90 and gln91), glul53 and ser155.

Alterations in these amino acid residues, either singly or in combination, may alter the biological activity of OPG. For example, changes in specific cysteine residues may alter the structure of individual

cysteine-rich domains, whereas changes in residues
important for ligand binding may affect physical
interactions of OPG with ligand. Structural models can
aid in identifying analogs which have more desirable
5   properties, such as enhanced biological activity,
greater stability, or greater ease of formulation.

A skilled artisan will appreciate that initially
sites should be modified by substitution in a
relatively conservative manner. Such conservative
10   substitutions are shown in Table 1 under the heading of
"Preferred Substitutions". If such substitutions result
in a change in biological activity, then more
substantial changes (Exemplary Substitutions) may be
introduced and/or other additions/deletions may be made
15   and the resulting products screened.

TABLE 1: Amino Acid Substitutions

Original Residue	Preferred Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Lys; Arg
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; norleucine
Leu (L)	Ile	norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Leu	Leu; Val; Ile; Ala
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; norleucine

In making such changes, the hydropathic index of amino acids may be considered. The importance of the
5   hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle (1982), J.

Mol. Biol., 157:105-131, the disclosure of which is
incorporated herein by reference). It is known that
certain amino acids may be substituted for other amino
acids having a similar hydropathic index or score and
5   still retain a similar biological activity.

It is also understood in the art that the
substitution of like amino acids can be made
effectively on the basis of hydrophilicity,
particularly where the functionally equivalent protein
10   or peptide thereby created is intended for use in
immunological embodiments, as in the present case. U.S.
Patent 4,554,101, the disclosure of which is
incorporated herein by reference, states that the
greatest local average hydrophilicity of a protein, as
15   governed by the hydrophilicity of its adjacent amino
acids, correlates with its immunogenicity and
antigenicity, i.e., with a biological property of the
protein.

U.S. Patent 4,554,101 also teaches the
20   identification and preparation of epitopes from primary
amino acid sequences on the basis of hydrophilicity.
Through the methods disclosed in U.S. Patent 4,554,101
a skilled artisan would be able to identify epitopes,
for example, within the amino acid sequence of an
25   sTNFR. These regions are also referred to as "epitopic
core regions". Numerous scientific publications have
been devoted to the prediction of secondary structure,
and to the identification of epitopes, from analyses of
amino acid sequences (Chou and Fasman (1974),
30   Biochemistry, 13(2):222-245; Chou and Fasman (1974),
Biochemistry, 13(2):211-222; Chou and Fasman (1978),
Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148; Chou
and Fasman (1978), Ann. Rev. Biochem., 47:251-276 and
Chou and Fasman (1979), Biophys. J., 26:367-384, the
35   disclosures of which are incorporated herein by
reference). Moreover, computer programs are currently

available to assist with predicting antigenic portions
and epitopic core regions of proteins. Examples include
those programs based upon the Jameson-Wolf analysis
(Jameson and Wolf (1988), Comput. Appl. Biosci.,
5   4(1):181-186 and Wolf et al. (1988), Comput. Appl.
Biosci., 4(1):187-191, the disclosures of which are
incorporated herein by reference); the program PepPlot®
(Brutlag et al. (1990), CABS, 6:237-245 and Weinberger
et al. (1985), Science, 228:740-742, the disclosures of
10   which are incorporated herein by reference); and other
programs for protein tertiary structure prediction
(Fetrow and Bryant (1993), BIOTECHNOLOGY, 11:479-483,
the disclosure of which is incorporated herein by
reference).

15   In contrast, substantial modifications in the
functional and/or chemical characteristics of a parent
molecule may be accomplished by selecting substitutions
that differ significantly in their effect on
maintaining (a) the structure of the polypeptide
20   backbone in the area of the substitution, for example,
as a sheet or helical conformation, (b) the relative
charge or hydrophobicity of the protein at the target
site or (c) the bulk of the side chain. Naturally-
occurring residues are divided into groups based on
25   common side chain properties:
          1) hydrophobic: norleucine, Met, Ala, Val, Leu,
             Ile;
          2) neutral hydrophilic: Cys, Ser, Thr;
          3) acidic: Asp, Glu;
30   4) basic: Asn, Gln, His, Lys, Arg;
          5) aromatic: Trp, Tyr, Phe; and
          6) residues that influence chain orientation:
             Gly, Pro.
          Non-conservative substitutions may involve the
35   exchange of a member of one of these groups for

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another. For example, substituted residues may be
introduced into regions of OPG or the sTNFRs that are
homologous with other NGF/TNF receptor family members
or into non-homologous regions of the protein.

5   A variety of amino acid substitutions or deletions
may be made to modify or add N-linked or O-linked
glycosylation sites, resulting in a protein with
altered glycosylation. The sequence may be modified to
add glycosylation sites to or to delete N-linked or O-

10   linked glycosylation sites from the parent molecule. An
asparagine-linked glycosylation recognition site
comprises a tripeptide sequence which is specifically
recognized by appropriate cellular glycosylation
enzymes. These tripeptide sequences are either

15   Asn-Xaa-Thr or Asn-Xaa-Ser, where Xaa can be any amino
acid other than Pro. In the 30 kDa TNF inhibitor, for
example, proven or predicted asparagine residues exist
at positions 14, 105 and 111.

        Specific mutations of the sequences of the parent

20   molecules may involve substitution of a non-native
amino acid at the amino-terminus, carboxy-terminus or
at any site of the protein that is modified by the
addition of an N-linked or O-linked carbohydrate. Such
modifications may be of particular utility in the

25   addition of an amino acid (e.g., cysteine), which is
advantageous for the linking of a water-soluble polymer
to form a derivative. For example, WO 92/16221
describes the preparation of sTNFR-I muteins, e.g.,
wherein an asparagine residue at position 105 of the

30   native human protein is changed to cysteine (c105
sTNFR-I).

        In a specific embodiment, a variant polypeptide
will preferably be substantially homologous to the
amino acid of the parent molecule from which it is

35   derived. The term "substantially homologous" as used
herein means a degree of homology that is in excess of

80%, preferably in excess of 90%, more preferably in
excess of 95% or most preferably even 99%. The
percentage of homology as described herein is
calculated as the percentage of amino acid residues
5   found in the smaller of the two sequences which align
with identical amino acid residues in the sequence
being compared when four gaps in a length of 100 amino
acids may be introduced to assist in that alignment, as
set forth by Dayhoff (1972), Atlas of Protein Sequence
10   and Structure, 5:124, National Biochemical Research
Foundation, Washington, D.C., the disclosure of which
is hereby incorporated by reference. Also included
within the term "substantially homologous" are
variant(s) of parent molecules that may be isolated by
15   cross-reactivity with antibodies to the parent molecule
amino acid sequences or whose genes may be isolated
through hybridization with the DNA of parent molecules
or segments thereof.

Polypeptide Derivatives
20   This invention also comprises chemically modified
derivatives of the parent molecule(s) in which the
protein is linked to a nonproteinaceous moiety (e.g., a
polymer) in order to modify properties. These
chemically modified parent molecules are referred to
25   herein as "derivatives". Such derivatives may be
prepared by one skilled in the art given the
disclosures herein. Conjugates may be prepared using
glycosylated, non-glycosylated or de-glycosylated
parent molecule(s) and suitable chemical moieties.
30   Typically non-glycosylated parent molecules and water-
soluble polymers will be used. Other derivatives
encompassed by the invention include post-translational
modifications (e.g., N-linked or O-linked carbohydrate
chains, processing of N-terminal or C-terminal ends),
35   attachment of chemical moieties to the amino acid
backbone, and chemical modifications of N-linked or O-

linked carbohydrate chains. The polypeptides may also
be modified with a detectable label, such as an
enzymatic, fluorescent, isotopic or affinity label to
allow for detection and isolation of the protein.

5   Water-soluble polymers are desirable because the
protein to which each is attached will not precipitate
in an aqueous environment, such as a physiological
environment. Preferably, the polymer will be
pharmaceutically acceptable for the preparation of a
10   therapeutic product or composition. One skilled in the
art will be able to select the desired polymer based on
such considerations as whether the polymer/protein
conjugate will be used therapeutically and, if so, the
therapeutic profile of the protein (e.g., duration of
15   sustained release; resistance to proteolysis; effects,
if any, on dosage; biological activity; ease of
handling; degree or lack of antigenicity and other
known effects of a water-soluble polymer on a
therapeutic proteins).

20   Suitable, clinically acceptable, water-soluble
polymers include but are not limited to polyethylene
glycol (PEG), polyethylene glycol propionaldehyde,
copolymers of ethylene glycol/propylene glycol,
monomethoxy-polyethylene glycol,
25   carboxymethylcellulose, dextran, polyvinyl alcohol
(PVA), polyvinyl pyrrolidone, poly-1, 3-dioxolane,
poly-1,3,6-trioxane, ethylene/maleic anhydride
copolymer, poly (β-amino acids) (either homopolymers or
random copolymers), poly(n-vinyl
30   pyrrolidone)polyethylene glycol, polypropylene glycol
homopolymers (PPG) and other polyalkylene oxides,
polypropylene oxide/ethylene oxide copolymers,
polyoxyethylated polyols (POG) (e.g., glycerol) and
other polyoxyethylated polyols, polyoxyethylated
35   sorbitol, or polyoxyethylated glucose, colonic acids or

other carbohydrate polymers, Ficoll or dextran and
mixtures thereof. As used herein, polyethylene glycol
is meant to encompass any of the forms that have been
used to derivatize other proteins, such as mono-(C1-
5   C10) alkoxy- or aryloxy-polyethylene glycol.
Polyethylene glycol propionaldehyde may have advantages
in manufacturing due to its stability in water.

         The water-soluble polymers each may be of any
molecular weight and may be branched or unbranched.
10   Generally, the higher the molecular weight or the more
branches, the higher the polymer:protein ratio. The
water-soluble polymers each typically have an average
molecular weight of between about 2 kDa to about 100
kDa (the term "about" indicating that in preparations
15   of a water-soluble polymer, some molecules will weigh
more, some less, than the stated molecular weight). The
average molecular weight of each water-soluble polymer
preferably is between about 5 kDa and about 40
kDa, more preferably between about 10kDa and about 35
20   kDa and most preferably between about 15kDa and about
30 kDa.

         There are a number of attachment methods available
to those skilled in the art, including acylation
reactions or alkylation reactions (preferably to
25   generate an amino-terminal chemically modified protein)
with a reactive water-soluble molecule. See, for
example, EP 0 401 384; Malik et al. (1992), Exp.
Hematol., 20:1028-1035; Francis (1992), Focus on Growth
Factors, 3(2):4-10, published by Mediscript, Mountain
30   Court, Friern Barnet Lane, London N20 OLD, UK; EP 0 154
316; EP 0 401 384; WO 92/16221; WO 95/34326; WO
95/13312; WO 96/11953; WO 96/19459 and WO 96/19459 and
the other publications cited herein that relate to
pegylation, the disclosures of which are hereby
35   incorporated by reference.

Pegylation also may be specifically carried out
using water-soluble polymers having at least one
reactive hydroxy group (e.g. polyethylene glycol). The
water-soluble polymer can be reacted with an activating
5   group, thereby forming an "activated linker" useful in
modifying various proteins. The activated linkers can
be monofunctional, bifunctional, or multifunctional.

Activating groups which can be used to link the
water-soluble polymer to two or more proteins include
10 the following: sulfone, maleimide, sulfhydryl, thiol,
triflate, tresylate, azidirine, oxirane and 5-pyridyl.
Useful reagents having a reactive sulfone group that
can be used in the methods include, without limitation,
chlorosulfone, vinylsulfone and divinylsulfone. These
15 PEG derivatives are stable against hydrolysis for
extended periods in aqueous environments at pHs of
about 11 or less, and can form linkages with molecules
to form conjugates which are also hydrolytically
stable. Useful homobifunctional derivatives are PEG-
20 bis-chlorosulfone and PEG-bis-vinylsulfone (see WO
95/13312).

WO 97/04003, the disclosure of which is hereby
incorporated by reference, teaches methods of making
sulfone-activated linkers by obtaining a compound
25 having a reactive hydroxyl group and converting the
hydroxyl group to a reactive Michael acceptor to form
an activated linker, with tetrahydrofuran as the
solvent for the conversion. The application also
teaches a process for purifying the activated linkers
30 which utilizes hydrophobic interaction chromatography
to separate the linkers based on size and end-group
functionality.

As an example, chemically modified derivatives of
OPG may provide such advantages as increased stability,
35 increased time in circulation, or decreased
immunogenicity (see U.S. Patent No. 4,179,337). The

chemical moieties for derivitization may be selected
from water-soluble polymers such as polyethylene
glycol, ethylene glycol/propylene glycol copolymers,
carboxymethylcellulose, dextran, polyvinyl alcohol and
5   the like. The polypeptides may be modified at random
positions within the molecule, or at predetermined
positions within the molecule and may include one, two,
three or more attached chemical moieties.

    One may specifically desire N-terminally
10 chemically modified protein. Using polyethylene glycol
as an illustration of the present compositions, one may
select from a variety of polyethylene glycol molecules
(by molecular weight, branching, etc.), the proportion
of polyethylene glycol molecules to protein (or
15 peptide) molecules in the reaction mix, the type of
pegylation reaction to be performed, and the method of
obtaining the selected N-terminally pegylated protein.
The method of obtaining the N-terminally pegylated
preparation (i.e., separating this moiety from other
20 monopegylated moieties if necessary) may be by
purification of the N-terminally pegylated material
from a population of pegylated protein molecules.
Selective N-terminal chemical modification may be
accomplished by reductive alkylation which exploits
25 differential reactivity of different types of primary
amino groups (lysine versus the N-terminal) available
for derivatization in a particular protein. Under the
appropriate reaction conditions, substantially
selective derivatization of the protein at the N-
30 terminus with a carbonyl group containing polymer is
achieved.

Polyvalent Forms

    Polyvalent forms, i.e., molecules comprising more
than one active moiety, may be constructed. In one
35 embodiment, an sTNFR variant may possess multiple tumor
necrosis factor binding sites for the TNF ligand.

Additionally, the molecule may possess at least one
tumor necrosis factor binding site and, depending upon
the desired characteristic of polyvalent form, at least
one site of another molecule (e.g., a TNF-α
5   inhibitor(s), and an OPG).

       Active moieties may be linked using conventional
coupling techniques (see WO 92/16221, WO 95/13312 and
WO 95/34326, the disclosures of which are hereby
incorporated by reference). For example, WO 92/16221
10   and WO 95/34326 describe the preparation of various
dimerized sTNFR-I molecules, e.g., dimerized c105
sTNFR-I. Techniques for formation of polyvalent forms
include photochemical crosslinking (e.g., exposure to
ultraviolet light), chemical crosslinking (e.g., with
15   bifunctional linker molecules such as polyethylene
glycol), and mutagenesis (e.g., introduction of
additional cysteine residues).

       Polyvalent forms may be constructed by chemically
coupling at least one parent molecule and another
20   moiety with any clinically accepted linker (e.g., a
water-soluble polymer). In principle, the linker must
not impart new immunogenicity. The linker also must
not, by virtue of the new amino acid residues, alter
the hydrophobicity and charge balance of the structure,
25   which affects its biodistribution and clearance. A
variety of chemical crosslinkers may be used depending
upon which properties of the protein dimer are desired.
For example, crosslinkers may be short and relatively
rigid or longer and more flexible, may be biologically
30   reversible, and may provide reduced immunogenicity or
longer pharmacokinetic half-life.

       In one example, OPG molecules are linked through
the amino terminus by a two step synthesis (see Example
12). In the first step, OPG is chemically modified at
35   the amino terminus to introduce a protected thiol,

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which after purification is deprotected and used as a
point of attachment for site-specific conjugation
through a variety of crosslinkers with a second OPG
molecule. Amino-terminal crosslinks include, but are
5   not limited to, a disulfide bond, thioether linkages
using short-chain, bis-functional aliphatic
crosslinkers, and thioether linkages to variable
length, bifunctional polyethylene glycol crosslinkers
(PEG "dumbbells"). Also encompassed by PEG dumbbell
10 synthesis of OPG dimers is a byproduct of such
synthesis, termed a "monobell". An OPG monobell
consists of a monomer coupled to a linear bifunctional
PEG with a free polymer terminus. Alternatively, OPG
may be crosslinked directly through a variety of amine
15 specific homobifunctional crosslinking techniques which
include reagents such as: diethylenetriaminepentaacetic
dianhydride (DTPA), p-benzoquinone (pBQ) or
bis(sulfosuccinimidyl) suberate (BS^3) as well as others
known in the art. It is also possible to thiolate OPG
20 directly with reagents such as iminothiolane in the
presence of a variety of bifunctional, thiol specific
crosslinkers, such as PEG bismaleimide, and achieve
dimerization and/or dumbbells in a one step process.
        The water-soluble polymers for this polyvalent
25 form can be, based on the monomers listed herein,
homopolymers, random or block copolymers, terpolymers
straight chain or branched, substituted or
unsubstituted. The polymer can be of any length or
molecular weight, but these characteristics can affect
30 the biological properties. Polymer average molecular
weights particularly useful for decreasing clearance
rates in pharmaceutical applications are in the range
of 2,000 to 35,000 daltons. In addition, the length of
the polymer can be varied to optimize or confer the
35 desired biological activity.

Alternatively, a bivalent molecule may consist of
two tandem repeats of parent molecules separated by a
polypeptide linker region. The design of the
polypeptide linkers is similar in design to the
5   insertion of short loop sequences between domains in
the de novo design of proteins (Mutter (1988), TIBS,
13:260-265 and Regan and DeGrado (1988), Science,
241:976-978, the disclosures of which are hereby
incorporated by reference). Several different linker
10 constructs have been assembled and shown to be useful
for forming single chain antibodies; the most
functional linkers vary in size from 12 to 25 amino
acids (amino acids having unreactive side groups, e.g.,
alanine, serine and glycine) which together constitute
15 a hydrophilic sequence, have a few oppositely charged
residues to enhance solubility and are flexible
(Whitlow and Filpula (1991), Methods: A Companion to
Methods in Enzymology, 2:97-105; and Brigido et al.
(1993), J. Immunol., 150:469-479, the disclosures of
20 which are hereby incorporated by reference). It has
been shown that a linker suitable for single chain
antibodies is effective to produce a dimeric form of
the human sTNFR-II (Neve et al. (1996), Cytokine,
8(5):365-370, the disclosure of which is hereby
25 incorporated by reference).

Self-associating variants are another example of
polyvalent forms. Such self-associating variants may be
bound covalently (typically by disulfide bonds) or
noncovalently. Analysis of carboxy-terminal deletions
30 of OPG, for example, suggest that at least a portion of
the region 186-401 is involved in association of OPG
polypeptides. Substitution of part or all of the region
of OPG amino acids 186-401 with an amino acid sequence
capable of self-association is also encompassed by the
35 invention.

Polyvalent forms may also be formed using substitution variants. Parent molecules may be modified to form dimers or multimers by site-directed mutagenesis to create unpaired cysteine residues for
5   interchain disulfide bond formation.

Additionally, a parent molecule may be chemically coupled to biotin, and the resulting conjugate may then be allowed to bind to avidin, resulting in tetravalent avidin/biotin/parent molecules. A parent molecule may
10 also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates.

In yet another embodiment, recombinant fusion
15 proteins may also be produced wherein each recombinant chimeric molecule has a parent molecule(s) sequence amino-terminally or carboxy-terminally fused to all or part of the constant domains, but at least one constant domain, of the heavy or light chain of human
20 immunoglobulin. For example, a chimeric TNF-α inhibitor(s)/IgG1 (or IgG1/TNF-α inhibitor(s)) fusion protein may be produced from a light chain-containing chimeric gene: a TNF-α inhibitor(s)/human kappa light chain chimera (TNF-α inhibitor(s)/Ck) or a human kappa
25 light chain/TNF-α inhibitor(s) chimera (Ck/TNF-α inhibitor(s)); or a heavy chain-containing chimeric gene: a TNF-α inhibitor(s)/human gamma-1 heavy chain chimera (TNF-α inhibitor(s)/Cg-1) or a human gamma-1 heavy chain/TNF-α inhibitor(s) chimera (Cg-1/TNF-α
30 inhibitor(s)). Alternatively, an OPG-Fc chimera may be formed as described in WO 97/23614, which is hereby incorporated by reference. Following transcription and translation of a heavy-chain chimeric gene, or of a light chain-containing gene and a heavy-chain chimeric

gene, the gene products may be assembled into a single
chimeric molecule having a parent molecule(s) displayed
bivalently. Additional details relating to the
construction of such chimeric molecules are disclosed
5   in United States Patent 5,116,964, WO 89/09622,
WO 91/16437, WO 97/23614 and EP 315062, the disclosures
of which are hereby incorporated by reference.

In yet a further embodiment, recombinant fusion
proteins may also be produced wherein each recombinant
10 chimeric molecule has at least one TNF-α inhibitor(s),
as described herein, and at least a portion of the
region 186-401 of osteoprotogerin or a variant thereof,
as described in European Patent Application No.
96309363.8, the disclosures of which are hereby
15 incorporated by reference. Either the TNF-α
inhibitor(s) or the portion of osteoprotogerin may be
at the amino-terminus or the carboxy-terminus of the
chimeric molecule.

Nucleic Acids
20   The invention provides for an isolated nucleic
acid encoding a polypeptide having at least one of the
biological activities of OPG. As described herein, the
biological activities of OPG include, but are not
limited to, any activity involving bone metabolism and
25 in particular, include increasing bone density. The
nucleic acids of the invention are selected from the
following:

a) the nucleic acid sequences as shown in Figures
2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D
30 (SEQ ID NO:124) or complementary strands thereof;

b) the nucleic acids which hybridize under
stringent conditions with the polypeptide-encoding
region in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID
NO:122), and 9C-9D (SEQ ID NO:124); and

c) nucleic acids which hybridize under stringent
conditions with nucleotides 148 through 337 inclusive
as shown in Figure 1A.

d) the nucleic acid sequences which are degenerate
5   to the sequences in (a) and (b).

The invention provides for nucleic acids which
encode rat, mouse and human OPG as well as nucleic acid
sequences hybridizing thereto which encode a
polypeptide having at least one of the biological
10 activities of OPG. Also provided for are nucleic acids
which hybridize to a rat OPG EST encompassing
nucleotides 148-337 as shown in Figure 1A. The
conditions for hybridization are generally of high
stringency such as 5xSSC, 50% formamide and 42°C
15 described in Example 1 of the specification. Equivalent
stringency to these conditions may be readily obtained
by adjusting salt and organic solvent concentrations
and temperature. The nucleic acids in (b) encompass
sequences encoding OPG-related polypeptides which do
20 not undergo detectable hybridization with other known
members of the TNF receptor superfamily. In a preferred
embodiment, the nucleic acids are as shown in Figures
2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D
(SEQ ID NO:124).

25   The length of hybridizing nucleic acids of the
invention may be variable since hybridization may occur
in part or all of the polypeptide-encoding regions as
shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B (SEQ ID
NO:122), and 9C-9D (SEQ ID NO:124), and may also occur
30 in adjacent noncoding regions. Therefore, hybridizing
nucleic acids may be truncations or extensions of the
sequences shown in Figures 2B-2C (SEQ ID NO:120), 9A-9B
(SEQ ID NO:122), and 9C-9D (SEQ ID NO:124). Truncated
or extended nucleic acids are encompassed by the
35 invention provided they retain one or more of the
biological properties of OPG. The hybridizing nucleic

acids may also include adjacent noncoding regions which
are 5' and/or 3' to the OPG coding region. The
noncoding regions include regulatory regions involved
in OPG expression, such as promoters, enhance,
5   translational initiation sites, transcription
termination sites and the like.

Hybridization conditions for nucleic acids are
described in Sambrook et al., Molecular Cloning: A
Laboratory Manual, 2" ed. Cold Spring Harbor Laboratory
10 Press, Cold Spring Harbor, New York (1989)

DNA encoding rat OPG was provided in plasmid pMO-
B1.1 deposited with the American Type Culture
Collection, Rockville, MD on December 27, 1995 under
ATCC accession no. 69970. DNA encoding mouse OPG was
15 provided in plasmid pRcCMV-murine OPG deposited with
the American Type Culture Collection, Rockville, MD on
December 27, 1995 under accession no. 69971. DNA
encoding human OPG was provided in plasmid pRcCMV -
human OPG deposited with the American Type Culture
20 Collection, Rockville, MD on December 27, 1995 under
accession no. 69969. The nucleic acids of the invention
will hybridize under stringent conditions to the DNA
inserts of ATCC accession nos. 69969, 69970, and 69971
and have at least one of the biological activities of
25 OPG.

Also provided by the invention are derivatives of
the nucleic acid sequences as shown in Figures 2B, 9A
and 9B. As used herein, derivatives include nucleic
acid sequences having addition, substitution, insertion
30 or deletion of one or more residues such that the
resulting sequences encode polypeptides having one or
more amino acid residues which have been added,
deleted, inserted or substituted and the resulting
polypeptide has the activity of OPG. The nucleic acid
35 derivatives may be naturally occurring, such as by
splice variation or polymorphism, or may be constructed

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using site-directed mutagenesis techniques available to
the skilled worker. One example of a naturally
occurring variant of OPG is a nucleic acid encoding a
lys to asn change at residue 3 within the leader
5   sequence (see Example 5). It is anticipated that
nucleic acid derivatives will encode amino acid changes
in regions of the molecule which are least likely to
disrupt biological activity. Other derivatives include
a nucleic acid encoding a membrane-bound form of OPG
10   having an extracellular domain as shown in Figures 2B-
2C (SEQ ID NO:120), 9A-9B (SEQ ID NO:122), and 9C-9D
(SEQ ID NO:124) along with transmembrane and
cytoplasmic domains.

In one embodiment, derivatives of OPG include
15   nucleic acids encoding truncated forms of OPG having
one or more amino acids deleted from the carboxy
terminus. Nucleic acids encoding OPG may have from 1 to
216 amino acids deleted from the carboxy terminus.
Optionally, an antibody Fc region may extend from the
20   new carboxy terminus to yield a biologically active
OPG-Fc fusion polypeptide. (see Example 11). In
preferred embodiments, nucleic acids encode OPG having
the amino acid sequence from residues 22-185, 22-189,
22-194 or 22-201 (using numbering in Figure 9E-F) and
25   optionally, encoding an Fc region of human IgG.

Also included are nucleic acids encoding truncated
forms of OPG having one or more amino acids deleted
from the amino terminus. Truncated forms include those
lacking part or all the 21 amino acids comprising the
30   leader sequence. Additionally, the invention provides
for nucleic acids encoding OPG having from 1 to 10
amino acids deleted from the mature amino terminus (at
residue 22) and ,optionally, having from 1 to 216 amino
acids deleted from the carboxy terminus (at residue
35   401). Optionally, the nucleic acids may encode a
methionine residue at the amino terminus. Examples of

such OPG truncated polypeptides are described in
Example 8.

    Examples of the nucleic acids of the invention
include cDNA, genomic DNA, synthetic DNA and RNA. cDNA
5 is obtained from libraries prepared from mRNA isolated
from various tissues expressing OPG. In humans, tissue
sources for OPG include kidney, liver, placenta and
heart. Genomic DNA encoding OPG is obtained from
genomic libraries which are commercially available from
10 a variety of species. Synthetic DNA is obtained by
chemical synthesis of overlapping oligonucleotide
fragments followed by assembly of the fragments to
reconstitute part or all of the coding region and
flanking sequences (see U.S. Patent No. 4,695,623
15 describing the chemical synthesis of interferon genes).
RNA is obtained most easily by procaryotic expression
vectors which direct high-level synthesis of mRNA, such
as vectors using T7 promoters and RNA polymerase.

    Nucleic acid sequences of the invention are used
20 for the detection of OPG sequences in biological
samples in order to determine which cells and tissues
are expressing OPG mRNA. The sequences may also be used
to screen cDNA and genomic libraries for sequences
related to OPG. Such screening is well within the
25 capabilities of one skilled in the art using
appropriate hybridization conditions to detect
homologus sequences. The nucleic acids are also useful
for modulating the expression of OPG levels by anti-
sense therapy or gene therapy. The nucleic acids are
30 also used for the development of transgenic animals
which may be used for the production of the polypeptide
and for the study of biological activity (see Example
3).

Vectors and Host Cells

35   Expression vectors containing nucleic acid
sequences encoding OPG, host cells transformed with

said vectors and methods for the production of OPG are
also provided by the invention. An overview of
expression of recombinant proteins is found in <u>Methods</u>
<u>of Enzymology</u> v. 185, Goeddel, D.V. ed. Academic Press
5   (1990).

Host cells for the production of OPG include
procaryotic host cells, such as <u>E</u>. <u>coli</u>, yeast, plant,
insect and mammalian host cells. <u>E</u>. <u>coli</u> strains such
as HB101 or JM101 are suitable for expression.
10   Preferred mammalian host cells include COS, CHOd-, 293,
CV-1, 3T3, baby hamster kidney (BHK) cells and others.
Mammalian host cells are preferred when post-
translational modifications, such as glycosylation and
polypeptide processing, are important for OPG activity.
15   Mammalian expression allows for the production of
secreted polypeptides which may be recovered from the
growth medium.

Vectors for the expression of OPG contain at a
minimum sequences required for vector propogation and
20   for expression of the cloned insert. These sequences
include a replication origin, selection marker,
promoter, ribosome binding site, enhancer sequences,
RNA splice sites and transcription termination site.
Vectors suitable for expression in the aforementioned
25   host cells are readily available and the nucleic acids
of the invention are inserted into the vectors using
standard recombinant DNA techniques. Vectors for
tissue-specific expression of OPG are also included.
Such vectors include promoters which function
30   specifically in liver, kidney or other organs for
production in mice, and viral vectors for the
expression of OPG in targeted human cells.

Using an appropriate host-vector system, OPG is
produced recombinantly by culturing a host cell
35   transformed with an expression vector containing
nucleic acid sequences encoding OPG under conditions

such that OPG is produced, and isolating the product of
expression. OPG is produced in the supernatant of
transfected mammalian cells or in inclusion bodies of
transformed bacterial host cells. OPG so produced may
5   be purified by procedures known to one skilled in the
art as described below. The expression of OPG in
mammalian and bacterial host systems is described in
Examples 7 and 8. Expression vectors for mammalian
hosts are exemplified by plasmids such as pDSRα
10   described in PCT Application No. 90/14363. Expression
vectors for bacterial host cells are exemplified by
plasmids pAMG21 and pAMG22-His described in Example 8.
Plasmid pAMG21 was deposited with the American Type
Culture Collection, Rockville, MD on July 24, 1996
15   under accession no. 98113. Plasmid pAMG22-His was
deposited with the American Type Culture Collection,
Rockville, MD on July 24, 1996 under accession no.
98112. It is anticipated that the specific plasmids and
host cells described are for illustrative purposes and
20   that other available plasmids and host cells could also
be used to express the polypeptides.
      The invention also provides for expression of OPG
from endogenous nucleic acids by in vivo or ex vivo
recombination events to allow modulation of OPG from
25   the host chromosome. Expression of OPG by the
introduction of exogenous regulatory sequences (e.g.
promoters or enhancers) capable of directing the
production of OPG from endogenous OPG coding regions is
also encompassed. Stimulation of endogenous regulatory
30   sequences capable of directing OPG production (e.g. by
exposure to transcriptional enhancing factors) is also
provided by the invention.

Antibodies

      Also encompassed by the invention are antibodies
35   specifically binding to OPG. Antigens for the
generation of antibodies may be full-length

polypeptides or peptides spanning a portion of the OPG
sequence. Immunological procedures for the generation
of polyclonal or monoclonal antibodies reactive with
OPG are known to one skilled in the art (see, for

5   example, Harlow and Lane, _Antibodies: A Laboratory
Manual Cold Spring Harbor Laboratory Press, Cold Spring
Harbor N.Y. (1988)). Antibodies so produced are
characterized for binding specificity and epitope
recognition using standard enzyme-linked immunosorbent

10   assays. Antibodies also include chimeric antibodies
having variable and constant domain regions derived
from different species. In one embodiment, the chimeric
antibodies are humanized antibodies having murine
variable domains and human constant domains. Also

15   encompassed are complementary determining regions
grafted to a human framework (so-called CDR-grafted
antibodies). Chimeric and CDR-grafted antibodies are
made by recombinant methods known to one skilled in the
art. Also encompassed are human antibodies made in

20   mice.

Anti-OPG antibodies of the invention may be used
as an affinity reagent to purify OPG from biological
samples (see Example 10). In one method, the antibody
is immobilized on CnBr-activated Sepharose and a column

25   of antibody-Sepharose conjugate is used to remove OPG
from liquid samples. Antibodies are also used as
diagnostic reagents to detect and quantitate OPG in
biological samples by methods described below.

Pharmaceutical compositions

30   The invention also provides for pharmaceutical
compositions comprising a therapeutically effective
amount of a polypeptide comprising OPG or the other
therapeutic molecules used (e.g., IL-1ra, sTNF-RI, or
SLPI) together with a pharmaceutically acceptable

35   diluent, carrier, solubilizer, emulsifier, preservative
and/or adjuvant. Two or more of the therapeutic

molecules (e.g., OPG, IL-1ra, sTNF-RI, or SLPI) can be
formulated together or packaged together in a kit. The
term "therapeutically effective amount" means an amount
which provides a therapeutic effect for a specified
5   condition and route of administration. The composition
may be in a liquid or lyophilized form and comprises a
diluent (Tris, acetate or phosphate buffers) having
various pH values and ionic strengths, solubilizer such
as Tween or Polysorbate, carriers such as human serum
10   albumin or gelatin, preservatives such as thimerosal or
benzyl alcohol, and antioxidants such as ascrobic acid
or sodium metabisulfite. Also encompassed are
compositions comprising any of the therapeutic
molecules modified with water-soluble polymers to
15   increase solubility or stability. Compositions may also
comprise incorporation of any of the therapeutic
molecules into liposomes, microemulsions, micelles or
vesicles for controlled delivery over an extended
period of time.
20   Specifically, compositions herein may comprise
incorporation into polymer matrices such as hydrogels,
silicones, polyethylenes, ethylene-vinyl acetate
copolymers, or biodegradable polymers. Examples of
hydrogels include polyhydroxyalkylmethacrylates (p-
25   HEMA), polyacrylamide, polymethacrylamide,
polyvinylpyrrolidone, polyvinyl alcohol and various
polyelectrolyte complexes. Examples of biodegradable
polymers include polylactic acid (PLA), polyglycolic
acid (PGA), copolymers of PLA and PGA, polyamides and
30   copolymers of polyamides and polyesters. Other
controlled release formulations include microcapsules,
microspheres, macromolecular complexes and polymeric
beads which may be administered by injection.
         Selection of a particular composition will depend
35   upon a number of factors, including the condition being
treated, the route of administration and the

pharmacokinetic parameters desired. A more extensive
survey of component suitable for pharmaceutical
compositions is found in Remington's Pharmaceutical
Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA
5   (1980).

     Compositions of the invention may be administered
by injection, either subcutaneous, intravenous or
intramuscular, or by oral, nasal, pulmonary or rectal
administration. The route of administration eventually
10   chosen will depend upon a number of factors and may be
ascertained by one skilled in the art.

     The invention also provides for pharmaceutical
compositions comprising a therapeutically effective
amount of the nucleic acids of the invention together
15   with a pharmaceutically acceptable adjuvant. Nucleic
acid compositions will be suitable for the delivery of
part or all of the therapeutic molecule coding region
to cells and tissues as part of an anti-sense or gene
therapy regimen.

20   Methods of Treatment

     Bone tissue provides support for the body and
consists of mineral (largely calcium and phosphorous),
a matrix of collagenous and noncollagenous proteins,
and cells. Three types of cells found in bone,
25   osteocytes, osteoblasts and osteoclasts, are involved
in the dynamic process by which bone is continually
formed and resorbed. Osteoblasts promote formation of
bone tissue whereas osteoclasts are associated with
resorption. Resorption, or the dissolution of bone
30   matrix and mineral, is a fast and efficient process
compared to bone formation and can release large
amounts of mineral from bone. Osteoclasts are involved
in the regulation of the normal remodeling of skeletal
tissue and in resorption induced by hormones. For
35   instance, resorption is stimulated by the secretion of
parathyroid hormone in response to decreasing

concentrations of calcium ion in extracellular fluids.
In contrast, inhibition of resorption is the principal
function of calcitonin. In addition, metabolites of
vitamin D alter the responsiveness of bone to
5   parathyroid hormone and calcitonin.

After skeletal maturity, the amount of bone in the
skeleton reflects the balance (or imbalance) of bone
formation and bone resorption. Peak bone mass occurs
after skeletal maturity prior to the fourth decade.
10 Between the fourth and fifth decades, the equilibrium
shifts and bone resorption dominates. The inevitable
decrease in bone mass with advancing years starts
earlier in females than males and is distinctly
accelerated after menopause in some females
15 (principally those of Caucasian and Asian descent).

Osteopenia is a condition relating generally to
any decrease in bone mass to below normal levels. Such
a condition may arise from a decrease in the rate of
bone synthesis or an increase in the rate of bone
20 destruction or both. The most common form of osteopenia
is primary osteoporosis, also referred to as
postmenopausal and senile osteoporosis. This form of
osteoporosis is a consequence of the universal loss of
bone with age and is usually a result of increase in
25 bone resorption with a normal rate of bone formation.
About 25 to 30 percent of all white females in the
United States develop symptomatic osteoporosis. A
direct relationship exists between osteoporosis and the
incidence of hip, femoral, neck and inter-trochanteric
30 fracture in women 45 years and older. Elderly males
develop symptomatic osteoporosis between the ages of 50
and 70, but the disease primarily affects females.

The cause of postmenopausal and senile
osteoporosis is unknown. Several factors have been
35 identified which may contribute to the condition. They
include alteration in hormone levels accompanying aging

and inadequate calcium consumption attributed to
decreased intestinal absorption of calcium and other
minerals. Treatments have usually included hormone
therapy or dietary supplements in an attempt to retard
5   the process. To date, however, an effective treatment
for bone loss does not exist.

The invention provides for a method of treating a
bone disorder using a therapeutically effective amount
of OPG. The bone disorder may be any disorder
10   characterized by a net bone loss (osteopenia or
osteolysis). In general, treatment with OPG is
anticipated when it is necessary to suppress the rate
of bone resorption. Thus treatment may be done to
reduce the rate of bone resorption where the resorption
15   rate is above normal or to reduce bone resorption to
below normal levels in order to compensate for below
normal levels of bone formation.

Conditions which are treatable with OPG include
the following:
20   • Osteoporosis, such as primary osteoporosis,
     endocrine osteoporosis (hyperthyroidism,
     hyperparathyroidism, Cushing's syndrome, and
     acromegaly), hereditary and congenital forms of
     osteoporosis (osteogenesis imperfecta,
25   homocystinuria, Menkes' syndrome, and Riley-Day
     syndrome) and osteoporosis due to immobilization
     of extremities.

     • Paget's disease of bone (osteitis deformans) in
     adults and juveniles

30   • Osteomyelitis, or an infectious lesion in bone,
     leading to bone loss.

     • Hypercalcemia resulting from solid tumors (breast,
     lung and kidney) and hematologic malignacies
     (multiple myeloma, lymphoma and leukemia),
35   idiopathic hypercalcemia, and hypercalcemia

associated with hyperthyroidism and renal function
disorders.

- Osteopenia following surgery, induced by steroid
  administration, and associated with disorders of
5   the small and large intestine and with chronic
      hepatic and renal diseases.
- Osteonecrosis, or bone cell death, associated with
  traumatic injury or nontraumatic necrosis
  associated with Gaucher's disease, sickle cell
10   anemia, systemic lupus erythematosus, rheumatoid
      arthritis, periodontal disease, osteolytic
      metastasis, and other conditions

      It is understood that OPG may be used alone or in
conjunction with other factors for the treatment of
15 bone disorders. In one embodiment, osteoprotegerin is
used in conjunction with a therapeutically effective
amount of a factor which stimulates bone formation.
Such factors include but are not limited to the bone
morphogenic factors designated BMP-1 through BMP-12;
20 transforming growth factor-β (TGF-β) and TGF-β family
members; interleukin-1 (IL-1) inhibitors; TNFα
inhibitors; parathyroid hormone and analogs thereof,
parathyroid related protein and analogs thereof; E
series prostaglandins; bisphosphonates (such as
25 alendronate and others); bone-enhancing minerals such
as fluoride and calcium; non-steroidal anti-
inflammatory drugs (NSAIDs), including COX-2
inhibitors, such as Celebrex™ and Vioxx™;
immunosuppressants, such as methotrexate or
30 leflunomide; serine protease inhibitors such as
secretory leukocyte protease inhibitor (SLPI); IL-6
inhibitors (e.g., antibodies to IL-6), IL-8 inhibitors
(e.g., antibodies to IL-8); IL-18 inhibitors (e.g., IL-
18 binding protein or IL-18 antibodies); Interleukin-1
35 converting enzyme (ICE) modulators; fibroblast growth

factors FGF-1 to FGF-10 and FGF modulators; PAF
antagonists; keratinocyte growth factor (KGF), KGF-
related molecules, or KGF modulators; matrix
metalloproteinase (MMP) modulators; Nitric oxide
5   synthase (NOS) modulators, including modulators of
inducible NOS; modulators of glucocorticoid receptor;
modulators of glutamate receptor; modulators of
lipopolysaccharide (LPS) levels; and noradrenaline and
modulators and mimetics thereof.

10   The invention also relates to treatment of IL-1
mediated disease by treatment with an IL-1 inhibitor in
conjunction with a serine protease inhibitor. In
particular, this method is useful for treatment of
asthma and rheumatoid arthritis.

15   The invention relates further to treatment of TNF-
mediated disease by treatment with a TNF inhibitor in
conjunction with a serine protease inhibitor. In
particular, this method is useful for treatment of
rheumatoid arthritis.

20   In preferred embodiments, a polypeptide comprising
OPG is used in conjunction with particular therapeutic
molecules to treat various inflammatory conditions,
autoimmune conditions, and other conditions leading to
bone loss. Depending on the condition and the desired
25   level of treatment, two, three, or more agents may be
administered. These agents may be provided together by
inclusion in the same formulation or inclusion in a
treatment kit, or they may be provided separately. When
administered by gene therapy, the genes encoding the
30   protein agents may be included in the same vector,
optionally under the control of the same promoter
region, or in separate vectors. Particularly preferred
molecules in the aforementioned classes are as follows.

- IL-1 inhibitors: IL-1ra proteins and soluble IL-1 receptors. The most preferred IL-1 inhibitor is anakinra.
- TNF-α inhibitors: soluble tumor necrosis factor receptor type I (sTNF-RI; -RI is also called the p55 receptor); soluble tumor necrosis factor receptor type II (also called the p75 receptor); and monoclonal antibodies that bind the TNF receptor. Most preferred is sTNF-RI as described in WO 98/24463, etanercept (Enbrel'), and Avakine'. Exemplary TNF-α inhibitors are described in EP 422 339, EP 308 378, EP 393 438, EP 398 327, and EP 418 014.
- serine protease inhibitors: SLPI, ALP, MPI, HUSI-I, BMI, and CUSI. These inhibitors also may be viewed as exemplary LPS modulators, as SLPI has been shown to inhibit LPS responses. Jin et al. (1997), Cell 88(3): 417-26 (incorporated by reference).

Particularly preferred methods of treatment concern use of TNF-α inhibitors and IL-1 inhibitors in conjunction with polypeptides comprising OPG. Such polypeptides may be used with either or both TNF-α inhibitors and IL-1 inhibitors for treatment of conditions such as rheumatoid arthritis and multiple sclerosis.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Identification and isolation of the rat OPG cDNA

Materials and methods for cDNA cloning and analysis are described in Maniatis et al, ibid. Polymerase chain reactions (PCR) were performed using a Perkin-Elmer 9600 thermocycler using PCR reaction mixture (Boehringer-Mannheim) and primer concentrations

specified by the manufacturer. In general, 25-50 μl
reactions were denatured at 94°C, followed by 20-40
cycles of 94°C for 5 seconds, 50-60°C for 5 seconds,
and 72°C for 3-5 minutes. Reactions were then analyzed

5   for 72 °C for 3-5 minutes. Reactions were then analyzed
by gel electrophoresis as described in Maniatis *et al.*,
ibid.

A cDNA library was constructed using mRNA isolated
from embryonic d20 intestine for EST analysis (Adams *et*

10   *al*. Science 252, 1651-1656 (1991)). Rat embryos were
dissected, and the entire developing small and large
intestine removed and washed in PBS. Total cell RNA was
purified by acid guanidinium thiocyanate-phenol-
chloroform extraction (Chomczynski and Sacchi Anal.

15   Biochem. 162, 156-159, (1987)). The poly (A+) mRNA
fraction was obtained from the total RNA preparation by
adsorption to, and elution from, Dynabeads Oligo (dT)25
(Dynal Corp) using the manufacturer's recommended
procedures. A random primed cDNA library was prepared

20   using the Superscript Plasmid System (Gibco BRL,
Gaithersburg, Md). The random cDNA primer containing
an internal Not I restriction site was used to initiate
first strand synthesis and had the following sequence:

      5'-AAAGGAAGGAAAAAAGCGGCCGCTACANNNNNNNNNT-3'

25   (SEQ ID NO:1)

                          Not I

For the first strand synthesis three separate
reactions were assembled that contained 2.5 μg of
poly(A) RNA and 120 ng, 360 ng or 1,080 ng of random

30   primer. After second strand synthesis, the reaction
products were separately extracted with a mixture of
phenol:choroform:isoamyl alcohol (25:24:1 ratio), and
then ethanol precipitated. The double strand (ds) cDNA
products of the three reactions were combined and

35   ligated to the following ds oligonucleotide adapter:

5'-TCGACCCACGCGTCCG-3' (SEQ ID NO:2)

3'-GGGTGCGCAGGCp-5' (SEQ ID NO:3)

After ligation the cDNA was digested to completion
with Not I, extracted with phenol:chloroform:isoamyl
5   (25:24:1) alcohol and ethanol precipitated. The
resuspended cDNA was then size fractionated by gel
filtration using premade columns provided with the
Superscript Plasmid System (Gibco BRL, Gaithersburg,
Md) as recommended by the manufacturer. The two
10   fractions containing the largest cDNA products were
pooled, ethanol precipitated and then directionally
ligated into Not I and Sal I digested pMOB vector DNA
(Strathmann et al, 1991). The ligated cDNA was
introduced into competent ElectroMAX DH10B E. coli
15   (Gibco BRL, Gaithersburg, MD) by electroporation. For
automated sequence analysis approximately 10,000
transformants were plated on 20cm x 20cm agar plates
containing ampicillin supplemented LB nutrient media.
The colonies that arose were picked and arrayed onto 96
20   well microtiter plates containing 200 ml of L-broth,
7.5% glycerol, and 50 µg/ml ampicillin. The cultures
were grown overnight at 37°C, a duplicate set of
microtiter plates were made using a sterile 96 pin
replicating tool, then both sets were stored at -80°C
25   for further analysis. For full-length cDNA cloning
approximately one million transformants were plated on
96 bacterial ampicillin plates containing about 10,000
clones each. The plasmid DNA from each pool was
separately isolated using the Qiagen Plasmid Maxi Kit
30   (Qiagen Corp.,Germany) and arrayed into 96 microtiter
plates for PCR analyses.

To sequence random fetal rat intestine cDNA
clones, glycerol stocks were thawed, and small aliquots
diluted 1:25 in distilled. Approximately 3.0 ul of
35   diluted bacterial cultures were added to PCR reaction

mixture (Boehringer-Mannheim) containing the following
oligonucleotides:

     5'-TGTAAAACGACGGCCAGT-3' (SEQ ID NO:4)

     5'-CAGGAAACAGCTATGACC-3' (SEQ ID NO:5)

5   The reactions were incubated in a thermocycler
(Perkin-Elmer 9600) with the following cycle
conditions: 94 C for 2 minutes; 30 cycles of 94°C for 5
seconds, 50°C for 5 seconds, and 72°C for 3 minutes.;
72°C for 4 minutes. After incubation in the
10 thermocycler, the reactions were diluted with 2.0 mL of
water. The amplified DNA fragments were further
purified using Centricon columns (Princeton
Separations) using the manufacturer's recommended
procedures. The PCR reaction products were sequenced on
15 an Applied Biosystems 373A automated DNA sequencer
using T3 primer (oligonucleotide 353-23; 5'-
CAATTAACCCTCACTAAAGG-3') (SEQ ID NO:6) Taq dye-
terminator reactions (Applied Biosystems) following the
manufacturer's recommended procedures.

20   The resulting 5' nucleotide sequence obtained
from randomly picked cDNA clones translated and then
compared to the existing database of known protein
sequences using a modified version of the FASTA
program (Pearson et al. Meth. Enzymol. 183, (1990)).
25 Translated sequences were also analysed for the
presence of a specific cysteine-rich protein motif
found in all known members of the tumor necrosis
factor receptor (TNFR) superfamily (Smith et al.
(1994) Cell 76: 959-62), using the sequence profile
30 method of Gribskov et al. (1987), Proc. Natl. Acad.
Sci. USA 83: 4355-9), as modified by Luethy et al.
(1994), Protein Science 3: 139-46.

     Using the FASTA and Profile search data, an EST,
FRI-1 (Fetal Rat Intestine-1), was identified as a
35 possible new member of the TNFR superfamily. FRI-1

contained an approximately 600 bp insert with a LORF of
about 150 amino acids. The closest match in the
database was the human type II TNFR (TNFR-II). The
region compared showed an about 43% homology between
5   TNFR-II and FRI-1 over this 150 aa LORF. Profile
analysis using the first and second cysteine-rich
repeats of the TNFR superfamily yielded a Z score of
about 8, indicating that the FRI-1 gene possibly
encodes a new family member.

10   To deduce the structure of the FRI-1 product, the
fetal rat intestine cDNA library was screened for full
length clones. The following oligonucleotides were
derived from the original FRI-1 sequence:

        5'-GCATTATGACCCAGAAACGGGAC-3' (SEQ ID NO:7)

15   5'-AGGTAGCGCCCTTCCTCACATTC-3' (SEQ ID NO:8)

        These primers were used in PCR reactions to screen
96 pools of plasmid DNA, each pool containing plasmid
DNA from 10,000 independent cDNA clones. Approximately
1 ug of plasmid pool DNA was amplified in a PCR
20   reaction mixture (Boehringer-Mannheim) using a Perkin-
Elmer 96 well thermal cycler with the following cycle
conditions: 2 min at 94°C, 1 cycle; 15 sec at 94°C, then
45 sec at 65°C, 30 cycles; 7 min at 65°C, 1 cycle. PCR
reaction products were analysed by gel electrophoresis.
25   13 out of 96 plasmid DNA pools gave rise to amplified
DNA products with the expected relative molecular mass.

        DNA from one positive pool was used to transform
competent ElectroMAX DH10B E. coli (Gibco BRL,
Gaithersburg, MD) as described above. Approximately
30   40,000 transformants were plated onto sterile
nitrocellulose filters (BA-85, Schleicher and Schuell),
and then screened by colony hybridization using a [32]P-
dCTP labeled version of the PCR product obtained above.
Filters were prehybridized in 5X SSC, 50% deionized
35   formamide, 5X Denhardt's solution, 0.5% SDS, and 100
ug/ml denatured salmon sperm DNA for 2-4 hours at 42°C.

Filters were then hybridized in 5X SSC, 50% deionized
formamide, 2X Denhardt's solution, 0.1% SDS, 100 µg/ml
denatured salmon sperm DNA, and about 5 ng/ml of
labelled probe for about 18 hours at 42°C. The filters
5   were then washed in 2X SSC for 10 min at RT, 1X SSC for
10 minutes at 55°C, and finally in 0.5X SSC for 10-15
min at 55°C. Hybridizing clones were detected following
autoradiography, and then replated onto nitrocellulose
filters for secondary screening. Upon secondary
10 screening, a plasmid clone (pB1.1) was isolated, then
amplified in L-broth media containing 100 ug/ml
ampicillin and the plasmid DNA obtained. Both strands
of the 2.4 kb pB1.1 insert were sequenced.

The pB1.1 insert sequence was used for a FASTA
15 search of the public database to detect any existing
sequence matches and/or similarities. No matches to any
known genes or EST's were found, although there was an
approximate 45% similarity to the human and mouse TNFR-
II genes. A methionine start codon is found at bp 124
20 of the nucleotide sequence, followed by a LORF encoding
401 aa residues that terminates at bp 1327. The 401 aa
residue product is predicted to have a hydrophobic
signal peptide of approximately 31 residues at its
N-terminus, and 4 potential sites of N-linked
25 glycosylation. No hydrophobic transmembrane spanning
sequence was identified using the PepPlot program
(Wisconsin GCG package, version 8.1). The deduced 401
aa sequence was then used to search the protein
database. Again, there were no existing matches,
30 although there appeared to be a strong similarity to
many members of the TNFR superfamily, most notably the
human and mouse TNFR-II. A sequence alignment of this
novel protein with known members of the TNFR-
superfamily was prepared using the Pileup program, and
35 then modified by PrettyPlot (Wisconsin GCG package,

version 8.1). This alignment shows a clear homology
between the full length FRI-1 gene product and all
other TNFR family members. The homologus region maps to
the extracellular domain of TNFR family members, and
5   corresponds to the three or four cysteine-rich repeats
found in the ligand binding domain of these proteins.
This suggested that the FRI-1 gene encoded a novel TNFR
family member. Since no transmembrane spanning region
was detected we predicted that this may be a secreted
10   receptor, similar to TNFR-I derived soluble receptors
(Kohno et al. (1990), Proc. Natl. Acad. Sci. USA 87:
8331-5). Due to the apparent biological activity of the
FRI-1 gene (vide infra), the product was named
Osteoprotegerin (OPG).

15   **EXAMPLE 2**
        OPG mRNA Expression Patterns in Tissues
        Multiple human tissue northern blots (Clonetech)
were probed with a ^{32}P-dCTP labelled FRI-1 PCR product
to detect the size of the human transcript and to
20   determine patterns of expression. Northern blots were
prehybridized in 5X SSPE, 50% formamide, 5X Denhardt's
solution, 0.5% SDS, and 100 µg/ml denatured salmon
sperm DNA for 2-4 hr at 42°C. The blots were then
hybridized in 5X SSPE, 50% formamide, 2X Denhardt's
25   solution, 0.1% SDS, 100 µg/ml denatured salmon sperm
DNA, and 5 ng/ml labelled probe for 18-24 hr at 42°C.
The blots were then washed in 2X SSC for 10 min at room
temperature, 1X SSC for 10 min at 50°C, then in 0.5X
SSC for 10-15 min.
30   Using a probe derived from the rat gene, a
predominant mRNA species with a relative molecular mass
of about 2.4 kb is detected in several tissues,
including kidney, liver, placenta, and heart. Highest
levels are detected in the kidney. A large mRNA species
35   of Mr 4.5 and 7.5 kb was detected in skeletal muscle

and pancreas. In human fetal tissue, kidney was found
to express relatively high levels of the 2.4 kb mRNA.
Using a human probe (vide infra), only the 2.4 kb
transcript is detected in these same tissues. In
5   addition, relatively high levels of the 2.4 kb
transcript was detected in the lymph node, thymus,
spleen and appendix. The size of the transcript
detected by both the rat and human Osteosprotegerin
gene is almost identical to the length of the rat pB1.1
10 FRI-1 insert, suggesting it was a full length cDNA
clone.

                        EXAMPLE 3
        Systemic delivery of OPG in transgenic mice
        The rat OPG clone pB1.1 was used as template to
15 PCR amplify the coding region for subcloning into an
ApoE-liver specific expression vector (Simonet _et al_.
J. Clin. Invest. <u>94</u>, 1310-1319 (1994), and PCT
Application No. US94/11675 and co-owned U.S. Serial No.
08/221,767. The following 5' and 3' oligonucleotide
20 primers were used for PCR amplification, respectively:
        5'-GACTAGTCCCACAATGAACAAGTGGCTGTG-3'
        (SEQ ID NO:9)
        5'-ATAAGAATGCGGCCGCTAAACTATGAAACAGCCCAGTGACCATTC-3'
        (SEQ ID NO:10)
25   The PCR reaction mixture (Boehringer-Mannheim) was
treated as follows: 94°C for 1 minute, 1 cycle; 94°C
for 20 sec, 62°C for 30 sec, and 74 C for 1 minute, 25
cycles. Following amplification, the samples were
purified over Qiagen PCR columns and digested overnight
30 with SpeI and NotI restriction enzymes. The digested
products were extracted and precipitated and subcloned
into the ApoE promoter expression vector. Prior to
microinjecting the resulting clone, HE-OPG, it was
sequenced to ensure it was mutation-free.
35   The HE-OPG plasmid was purified through two rounds
of CsCl density gradient centrifugation. The purified

plasmid DNA was digested with XhoI and Ase I, and the
3.6 kb transgene insert was purified by gel
electrophoresis. The purified fragment was diluted to a
stock injection solution of 1 µg/ml in 5 mM Tris, pH
5   7.4, 0.2 mM EDTA. Single-cell embryos from BDF1 x BDF1-
bred mice were injected essentially as described
(Brinster et al. (1985), Proc. Natl. Acad. Sci. USA 82:
4338), except that injection needles were beveled and
siliconized before use. Embryos were cultured overnight
10   in a CO_2 incubator and 15 to 20 2-cell embryos were
transferred to the oviducts of pseudopregnant CD1
female mice.
     Following term pregnancy, 49 offspring were
obtained from implantation of microinjected embryos.
15   The offspring were screened by PCR amplification of the
integrated transgene in genomic DNA samples. The target
region for amplification was a 369 bp region of the
human Apo E intron which was included in the expression
vector. The oligos used for PCR amplification were:
20   5'- GCC TCT AGA AAG AGC TGG GAC-3' (SEQ ID NO:11)
          5'- CGC CGT GTT CCA TTT ATG AGC-3' (SEQ ID NO:12)
     The conditions for PCR were: 94°C for 2 minute, 1
cycle; 94°C for 1 min, 63°C for 20 sec, and 72°C for 30
sec, 30 cycles. Of the 49 original offspring, 9 were
25   identified as PCR positive transgenic founders.
     At 8-10 weeks of age, five transgenic founders (2,
11, 16, 17, and 28) and five controls (1, 12, 15, 18,
and 30) were sacrificed for necropsy and pathological
analysis. Liver was isolated from the remaining 4
30   founders by partial hepatectomy. For partial
hepatectomy, the mice were anesthetized and a lobe of
liver was surgically removed. Total cellular RNA was
isolated from livers of all transgenic founders, and 5
negative control littermates as described (McDonald et
35   al. Meth. Enzymol. 152, 219 (1987)). Northern blot

analysis was performed on these samples to assess the
level of transgene expression. Approximately 10ug of
total RNA from each animal liver was resolved by
electrophoresis denaturing gels (Ogden et al. Meth.
5   Enzymol 152, 61 (1987)), then transferred to HYBOND-N
nylon membrane (Amersham), and probed with ^{32}P
dCTP-labelled pBl.1 insert DNA. Hybridization was
performed overnight at 42°C in 50% Formamide, 5 x SSPE,
0.5% SDS, 5 x Denhardt's solution, 100 µg/ml denatured
10   salmon sperm DNA and 2-4 x 10^6 cpm of labeled probe/ml
of hybridization buffer. Following hybridization, blots
were washed twice in 2 x SSC, 0.1% SDS at room
temperature for 5 min each, and then twice in 0.1 x
SSC, 0.1% SDS at 55°C for 5-10 min each. Expression of
15   the transgene in founder and control littermates was
determined following autoradiography.

        The northern blot data indicate that 7 of the
transgenic founders express detectable levels of the
transgene mRNA (animal #'s 2,11,16,17,22,33,and 45).
20   The negative control mice and one of the founders (#28)
expressed no transgene-related mRNA. Since OPG is
predicted to be a secreted protein, overexpression of
transgene mRNA should be a proxy for the level of
systemically delivered gene product. Of the PCR and
25   northern blot positive mice, animal 2, 17 and 22
expressed the highest levels of transgene mRNA, and may
show more extensive biological effects on host cells
and tissues.

                        EXAMPLE 4
30   Biological activity of OPG
        Five of the transgenic mice (animals 2,11,16,17
and 28) and 5 control littermates (animals 1,12,15,18,
and 30) were sacrificed for necropsy and pathological
analysis using the following procedures:

Prior to euthanasia, all animals had their
identification numbers verified, then were weighed,
anesthetized and blood drawn. The blood was saved as
both serum and whole blood for a complete serum
5   chemistry and hematology panel. Radiography was
performed just after terminal anesthesia by lethal CO_2
inhalation, and prior to the gross dissection.
Following this, tissues were removed and fixed in 10%
buffered Zn-Formalin for histological examination. The
10   tissues collected included the liver, spleen, pancreas,
stomach, duodenum, ileum, colon, kidney, reproductive
organs, skin and mammary glands, bone, brain, heart,
lung, thymus, trachea, eosphagus, thyroid, jejunem,
cecum, rectum, adrenals, urinary bladder, and skeletal
15   muscle. Prior to fixation the whole organ weights were
determined for the liver, stomach, kidney, adrenals,
spleen, and thymus. After fixation the tissues were
processed into paraffin blocks, and 3 um sections were
obtained. Bone tissue was decalcified using a formic
20   acid solution, and all sections were stained with
hematoxylin and eosin. In addition, staining with
Gomori's reticulin and Masson's trichrome were
performed on certain tissues. Enzyme histochemistry was
performed to determine the expression of tartrate
25   resistant acid phosphatase (TRAP), an enzyme highly
expressed by osteoclasts, multinucleated bone-resorbing
cells of monocyte-macrophage lineage.
Immunohistochemistry for BrdU and F480 monocyte-
macrophage surface antigen was also performed to detect
30   replicating cells and cells of the monocyte-macrophage
lineage, respectively. To detect F480 surface antigen
expression, formalin fixed, paraffin embedded 4µm
sections were deparaffinized and hydrated to deionized
water. The sections were quenched with 3% hydrogen
35   peroxide, blocked with Protein Block (Lipshaw,
Pittsburgh, PA), and incubated in rat monoclonal anti-

mouse F480 (Harlan, Indianapolis, IN). This antibody
was detected by biotinylated rabbit anti-rat
immunoglobulins, peroxidase conjugated strepavidin
(BioGenex San Ramon, CA) with DAB as chromagen (BioTek,
5   Santa Barbara, CA). Sections were counterstained with
hematoxylin.

        Upon gross dissection and observation of visceral
tissues, no abnormalities were found in the transgene
expressors or control littermates. Analysis of organ
10   weight indicate that spleen size increased by
approximately 38% in the transgenic mice relative to
controls. There was a slight enlargement of platelet
size and increased circulating unstained cells in the
transgene expressors. There was a marginal decrease in
15   platelet levels in the transgene expressors. In
addition, the serum uric acid, urea nitrogen, and
alkaline phosphatase levels all trended lower in the
transgene expressors. The expressors were found to have
increased radiodensity of the skeleton, including long
20   bones (femurs), vertebrae, and flat bones (pelvis). The
relative size of femurs in the expressors were not
different from the the control mice.

        Histological analysis of stained sections of bone
from the OPG expressors show severe osteopetrosis with
25   the presence of cartilage remnants from the primary
spongiosa seen within bone trabeculae in the diaphysis
of the femur. A clearly defined cortex was not
identifiable in the sections of femur. In normal
animals, the central diaphysis is filled with bone
30   marrow. Sections of vertebra also show osteopetrotic
changes implying that the OPG-induced skeletal changes
were systemic. The residual bone marrow showed
predominantly myeloid elements. Megakaryocytes were
present. Reticulin stains showed no evidence for
35   reticulin deposition. Immunohistochemistry for F480, a
cell surface antigen expressed by cells of monocyte-

macrophage derivation in the mouse, showed the presence
of F480 positive cells in the marrow spaces. Focally,
flattened F480 positive cells could be seen directly
adjacent to trabecular bone surfaces.

5   The mesenchymal cells lining the bony trabeculae
were flattened and appeared inactive. Based on H&E and
TRAP stains, osteoclasts were rarely found on the
trabecular bone surfaces in the OPG expressors. In
contrast, osteoclasts and/or chondroclasts were seen in

10   the region of the growth plate resorbing cartilage, but
their numbers may be reduced compared to controls.
Also, osteoclasts were present on the cortical surface
of the metaphysis where modelling activity is usually
robust. The predominant difference between the

15   expressors and controls was the profound decrease in
trabecular osteoclasts, both in the vertebrae and
femurs. The extent of bone accumulation was directly
correlated with the level of OPG transgene mRNA
detected by northern blotting of total liver RNA.

20   The spleens from the OPG expressors had an
increased amount of red pulp with the expansion due to
increased hematopoiesis. All hematopoietic lineages are
represented. F480 positive cells were present in both
control and OPG expressors in the red pulp. Two of the

25   expressors (2 and 17) had foci of extramedullary
hematopoiesis within the liver and this is likely due
to the osteopetrotic marrow.

There were no observable abnormalities in the
thymus, lymph nodes, gastrointestinal tract, pancreato-

30   hepatobiliary tract, respiratory tract, reproductive
system, genito-urinary system, skin, nervous system,
heart and aorta, breast, skeletal muscle and fat.

                      EXAMPLE 5
          Isolation of mouse and human OPG cDNA

35   A cDNA clone corresponding to the 5' end of the
mouse OPG mRNA was isolated from a mouse kidney cDNA

library (Clontech) by PCR amplification. The
oligonucleotides were derived from the rat OPG cDNA
sequence and are shown below:

5   5'-ATCAAAGGCAGGGCATACTTCCTG-3' (SEQ ID NO:13)
      5'-GTTGCACTCCTGTTTCACGGTCTG-3' (SEQ ID NO:14)
      5'-CAAGACACCTTGAAGGGCCTGATG-3' (SEQ ID NO:15)
      5'-TAACTTTTACAGAAGAGCATCAGC-3' (SEQ ID NO:16)

      5'-AGCGCGGCCGCCATGAACAAGTGGCTGGTGCTGCG-3' (SEQ ID NO:17)
10   5'-AGCTCTAGAGAAACAGCCCAGTGACCATTCC-3' (SEQ ID NO:18)

      The partial and full-length cDNA products obtained
in this process were sequenced. The full-length product
was digested with Not I and XbaI, then directionally
cloned into the plasmid vector pRcCMV (Invitrogen). The
15 resulting plasmid was named pRcCMV-Mu-OPG. The
nucleotide sequence of the cloned product was compared
to the rat OPG cDNA sequence. Over the 1300 bp region
spanning the OPG LORF, the rat and mouse DNA sequences
are approximately 88% identical. The mouse cDNA
20 sequence contained a 401 aa LORF, which was compared to
the rat OPG sequence and found to be about 94%
identical without gaps. This indicates that the mouse
cDNA sequence isolated encodes the murine OPG, and that
the sequence and structure has been highly conserved
25 throughout evolution. The mouse OPG sequence contains
an identical putative signal peptide at its N-terminus,
and all 4 potential sites of N-linked glycosylation are
conserved.

      A partial human OPG cDNA was cloned from a human
30 kidney cDNA library using the following rat-specific
oligonucleotides:

      5'-GTG AAG CTG TGC AAG AAC CTG ATG-3' (SEQ ID NO:19)

      5'-ATC AAA GGC AGG GCA TAC TTC CTG-3' (SEQ ID NO:20)

      This PCR product was sequenced and used to design
35 primers for amplifying the 3' end of the human cDNA
using a human OPG genomic clone in lambda as template:

      5'-TCCGTAAGAAACAGCCCAGTGACC-3' (SEQ ID NO:29)

5'-CAGATCCTGAAGCTGCTCAGTTTG-3' (SEQ ID NO:21)

The amplified PCR product was sequenced, and together with the 5' end sequence, was used to design 5' and 3' human-specific primers useful for amplifying

5 the entire human OPG cDNA coding sequences:

5'-AGCGCGGCCGCGGGGACCACAATGAACAAGTTG-3' (SEQ ID NO:22)

5'-AGCTCTAGAATTGTGAGGAAACAGCTCAATGGC-3' (SEQ ID NO:23)

The full-length human PCR product was sequenced, then directionally cloned into the plasmid vector

10 pRcCMV (Invitrogen) using Not I and Xba I. The resulting plasmid was named pRcCMV-human OPG. The nucleotide sequence of the cloned product was compared to the rat and mouse OPG cDNA sequences. Over the 1300 bp region spanning the OPG LORF, the rat and mouse DNA

15 sequences are approximately 78-88% identical to the human OPG cDNA. The human OPG cDNA sequence also contained a 401 aa LORF, and it was compared to the rat and mouse protein sequences. The predicted human OPG is approximatley 85% identical, and about 90% identical

20 to the rat and mouse proteins, respectively. Sequence alignment of rat, mouse and human proteins show that they have been highly conserved during evolution. The human protein is predicted to have a N-terminal signal peptide, and 5 potential sites of N-linked

25 glycosylation, 4 of which are conserved between the rat and mouse OPG.

The DNA and predicted amino acid sequence of mouse OPG is shown in Figure 9A and 9B (SEQ ID NO:122). The DNA and predicted amino acid sequence of human OPG is

30 shown in Figure 9C an 9D (SEQ ID NO:124). A comparison of the rat, mouse and human OPG amino acid sequences is shown in Figure 9E and 9F.

Isolation of additional human OPG cDNA clones revealed the presence of a G to C base change at

35 position 103 of the DNA sequence shown in Figure 9C. This nucleotide change results in substitution of an

asparagine for a lysine at position 3 of the amino acid
sequence shown in Figure 9C. The remainder of the
sequence in clones having this change was identical to
that in Figure 9C and 9D.

5   **EXAMPLE 6**

        OPG three-dimensional structure modelling

        The amino-terminal portion of OPG has homology to
the extracellular portion of all known members of the
TNFR superfamily (Figure 1C). The most notable motif in

10 this region of TNFR-related genes is an about 40 amino
acid, cysteine-rich repeat sequence which folds into
distinct structures (Banner et al. (1993), Cell 73:
431-45). This motif is usually displayed in four (range
3-6) tandem repeats (see Figure 1C), and is known to be

15 involved in ligand binding (Beutler and van Huffel
(1994), Science 264: 667-73). Each repeat usually
contains six interspaced cysteine residues, which are
involved in forming three intradomain disulfide bonds,
termed SS1, SS2, and SS3 (Banner et al., ibid). In some

20 receptors, such as TNFR2, CD30 and CD40, some of the
repeat domains contain only two intrachain disulfide
bonds (SS1 and SS3).

        The human OPG sequence was aligned to a TNFR1
extracellular domain profile using methods described by

25 Luethy, et al., ibid, and the results were graphically
displayed using the PrettyPlot program from the
Wisconsin Package, version 8.1 (Genetics Computer
Group, Madison, WI) (Figure 10). The alignment
indicates a clear conservation of cysteine residues

30 involved in formation of domains 1-4. This alignment
was then used to construct a three-dimensional (3-D)
model of the human OPG N-terminal domain using the
known 3-D structure of the extracellular domain of p55
TNFR1 (Banner et al., ibid) as the template. To do this

35 the atomic coordinates of the peptide backbone and side
chains of identical residues were copied from the

crystal structure coordinates of TNFR1. Following this,
the remaining coordinates for the insertions and
different side chains were generated using the LOOK
program (Molecular Applications Group, Palo Alto, CA).
5   The 3-D model was then refined by minimizing its
conformational energy using LOOK.

By analogy with other TNFR family members, it is
assumed that OPG binds to a ligand. For the purpose of
modelling the interaction of OPG with its ligand, the
10 crystal structure of TNF-β was used to simulate a 3-D
representation of an "OPG ligand". This data was
graphically displayed (see Figure 11) using Molscript
(Kraulis (1991), <u>J. Appl. Cryst.</u> 24: 946-50). A model
for the OPG/ligand complex with 3 TNFβ and 3 OPG
15 molecules was constructed where the relative positions
of OPG are identical to TNFR1 in the crystal structure.
This model was then used to find the residues of OPG
that could interact with its ligand using the following
approach: The solvent accessible area of all residues
20 in the complex and one single OPG model were
calculated. The residues that have different
accessibility in the complex than in the monomer are
likely to interact with the ligand.

The human and mouse OPG amino acid sequences were
25 realigned using this information to highlight sequences
comprising each of the cysteine rich domains 1-4
(Figure 12A and 12B). Each domain has individual
structural characteristics which can be predicted.

<u>Domain 1</u>: Contains 4 cysteines involved in SS2
30 (C41 to C54) and SS3 (C44 to C62) disulfide bonds.
Although no SS1 bond is evident based on disulfide
bridges, the conserved tyrosine at position 28 is
homologous to Y20 in TNFR1, which is known to be
involved in interacting with H66 to aid in domain
35 formation. OPG has a homologous histidine at position

75, suggesting OPG Y28 and H75 stack together in the
native protein, as do the homologous residues in TNFR1.
Therefore, both of these residues may indeed be
important for biological activity, and N-terminal OPG
5   truncations up to and beyond Y28 may have altered
activity. In addition, residues E34 and K43 are
predicted to interact with a bound ligand based on our
3-dimensional model.

Domain 2: Contains six cysteines and is predicted
10 to contain SS1 (C65 to C80), SS2 (C83 to C98) and SS3
(C87 to C105) disulfide bonds. This region of OPG also
contains an region stretching from P66-Q91 which aligns
to the portion of TNFR1 domain 2 which forms close
contacts with TNFβ (see above), and may interact with
15 an OPG ligand. In particular residues P66, H68, Y69,
Y70, T71, D72, S73, H75, T76, S77, D78, E79, L81, Y82,
P85, V86, K88, E89, L90, and Q91 are predicted to
interact with a bound ligand based on our structural
data.

20 Domain 3: Contains 4 cysteines involved in SS1
(C107 to C 118) and SS3 (C124 to C142) disulfide bonds,
but not an SS2 bond. Based on our structural data,
residues E115, L118 and K119 are predicted in to
interact with an OPG ligand.

25 Domain 4: Contains 4 cysteines involved in SS1
(C145 to C160) and SS3 (C166 to C185) disulfide bonds,
but not an SS2 bond, similar to domain 3. Our
structural data predict that E153 and S155 interact
with an OPG ligand.

30 Thus, the predicted structural model for OPG
identifies a number of highly conserved residues which
are likely to be important for its biological activity.

EXAMPLE 7

Production of recombinant secreted

35 #### OPG in mammalian cells

To determine if OPG is actually a secreted protein, mouse OPG cDNA was fused to the human IgG1 Fc domain as a tag (Capon et al. Nature 337, 525-531 (1989)), and expressed in human 293 fibroblasts. Fc
5   fusions were carried out using the vector pFc-A3. pFc-A3 contains the region encoding the Fc portion of human immunoglobulin IgG-γ1 heavy chain (Ellison et al. ibid) from the first amino acid of the hinge domain (Glu-99) to the carboxyl terminus and is flanked by a 5'-NotI
10 fusion site and 3'-SalI and XbaI sites. The plasmid was constructed by PCR amplification of the human spleen cDNA library (Clontech). PCR reactions were in a final volume of 100 μl and employed 2 units of Vent DNA polymerase (New England Biolabs) in 20 mM Tris-HCl (pH
15 8.8), 10 mM KCl, 10 μM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100 with 400 μM each dNTP and 1 ng of the cDNA library to be amplified together with 1 μM of each primer. Reactions were initiated by denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s,
20 55°C for 30 s, and 73°C for 2 min. The 5' primer
       5' ATAGCGGCCGCTGAGCCCAAATCTTGTGACAAAACTCAC 3'
       (SEQ ID NO:24)
incorporated a NotI site immediately 5' to the first residue (Glu-99) of the hinge domain of IgG-γ1. The 3'
25 primer
       5'-TCTAGAGTCGACTTATCATTTACCCGGAGACAGGGAGAGGCTCTT-3'
       (SEQ ID NO:25)
incorporated SalI and XbaI sites. The 717-bp PCR product was digested with NotI and SalI, isolated by
30 electrophoresis through 1% agarose (FMC Corp.),purified by the Geneclean procedure (BIO 101, Inc.) and cloned into NotI, SalI-digested pBluescript II KS vector (Stratagene). The insert in the resulting plasmid, pFc-A3, was sequenced to confirm the fidelity of the PCR
35 reaction.

The cloned mouse cDNA in plasmid pRcCMV-MuOPG was
amplified using the following two sets of primer pairs:

Pair 1:

5'-CCTCTGAGCTCAAGCTTCCGAGGACCACAATGAACAAG-3'

5   (SEQ ID NO:26)

5'-CCTCTGCGGCCGCTAAGCAGCTTATTTTCACGGATTGAACCTG-3'

(SEQ ID NO:27)

Pair 2:

5'-CCTCTGAGCTCAAGCTTCCGAGGACCACAATGAACAAG-3'

10   (SEQ ID NO:28)

5'-CCTCTGCGGCCGCTGTTGCATTTCCTTTCTG-3'

(SEQ ID NO:30)

The first pair amplifies the entire OPG LORF, and
creates a NotI restriction site which is compatible

15   with the in-frame Not I site in Fc fusion vector pFcA3.
pFcA3 was prepared by engineering a NotI restriction
site 5' to aspartic acid reside 216 of the human IgG1
Fc cDNA. This construct introduces a linker which
encodes two irrelevant amino acids which span the

20   junction between the OPG and IgG Fc region. This
product, when linked to the Fc portion, would encode
all 401 OPG residues directly followed by all 227 amino
acid residues of the human IgG1 Fc region (Fl.Fc). The
second primer pair amplifies the DNA sequences encoding

25   the first 180 amino acid residues of OPG, which
encompasses its putative ligand binding domain. As
above, the 3' primer creates an artificial Not I
restriction site which fuses the C-terminal truncated
OPG LORF at position threonine 180 directly to the IgG1

30   Fc domain (CT.fc).

The amino acid sequence junction linking OPG
residue 401 and aseptic acid residue 221 of the human
Fc region can be modified as follows: The DNA encoding
residues 216-220 of the human Fc region can be deleted

35   as described below, or the cysteine residue ·
corresponding to C220 of the human Fc region can be

mutated to either serine or alanine. OPF-Fc fusion
protein encoded by these modifed vectors can be
transfected into human 293 cells, or CHO cells, and
recombinant OPG-Fc fusion protein purified as described
5   below.

        Both products were directionally cloned into the
plasmid vector pCEP4 (Invitrogen). pCEP4 contains the
Epstein-Barr virus origin of replication, and is
capable of episomal replication in 293-EBNA-1 cells.
10 The parent pCEP4, and pCEP4-F1.Fc and pCEP4-CT.Fc
vectors were lipofected into 293-EBNA-1 cells using the
manufacturer's recommended methods. The transfected
cells were then selected in 100 µg/ml hygromycin to
select for vector expression, and the resulting drug-
15 resistant mass cultures were grown to confluence. The
cells were then cultured in serum-free media for 72 hr,
and the conditioned media removed and analysed by SDS-
PAGE. A silver staining of the polyacrylamide gel
detects the major conditioned media proteins produced
20 by the drug resistant 293 cultures. In the pCEP4-F1.Fc
and the pCEP4-CT.Fc conditioned media, unique bands of
the predicted sizes were abundantly secreted (see
Figures 13B and 13C). The full-length Fc fusion protein
accumulated to a high concentration, indicating that it
25 may be stable. Both Fc fusion proteins were detected by
anti-human IgG1 Fc antibodies (Pierce) on western
blots, indicating that they are recombinant OPG
products.

        The full length OPG-Fc fusion protein was purified
30 by Protein-A column chromatography (Pierce) using the
manufacturers recommended procedures. The protein was
then subjected to N-terminal sequence analysis by
automated Edman degradation as essentially described by
Matsudaira et al. (J. Biol. Chem. 262, 10-35 (1987)).

The following amino acid sequence was read after 19
cycles:

NH_2-E T L P P K Y L H Y D P E T G H Q L L-CO_2H
(SEQ ID NO:31)

5   This sequence was identical to the predicted mouse
OPG amino acid sequence beginning at amino acid residue
22, suggesting that the natural mammalian leader
cleavage site is between amino acid residues Q21-E22,
not between Y31-D32 as originally predicted.

10   expression experiments performed in 293-EBNA cells with
pCEP4-Fl.Fc and pCEP4-CT.Fc demonstrate that OPG is a
secreted protein, and may act systemically to bind its
ligand.

Procedures similar to those used to construct and
15   express the muOPG[22-180]-Fc and muOPG[22-401]-Fc
fusions were employed for additional mouse and human
OPG-Fc fusion proteins.

Murine OPG cDNA encoding amino acids 1-185 fused
to the Fc region of human IgG1 [muOPG Ct(185).Fc] was
20   constructed as follows. Murine OPG cDNA from plasmid
pRcCMV Mu Osteoprotegerin (described in Example 5) was
amplified using the following primer pair in a
polymerase chain reaction as described above:

1333-82:
25   5'-TCC CTT GCC CTG ACC ACT CTT-3'
(SEQ ID NO:32)

1333-80:
5'-CCT CTG CGG CCG CAC ACA CGT TGT CAT GTG TTG C-3'
(SEQ ID NO:33)

30   This primer pair amplifies the murine OPG cDNA
region encoding amino acid residues 63-185
(corresponding to bp 278-645) of the OPG reading frame
as shown in Figure 9A. The 3' primer contains a Not I
restriction site which is compatible with the in-frame

35   Not I site of the Fc fusion vector pFcA3. The product
also spans a unique EcoRI restriction site located at

bp 436. The amplified PCR product was purified, cleaved
with NotI and EcoRI, and the resulting EcoRI-NotI
restriction fragment was purified. The vector pCEP4
having the murine 1-401 OPG-Fc fusion insert was

5   cleaved with EcoRI and NotI, purified, and ligated to
the PCR product generated above. The resulting pCEP4-
based expression vector encodes OPG residues 1-185
directly followed by all 227 amino acid residues of the
human IgG1 Fc region. The murine OPG 1-185.Fc fusion

10 vector was transfected into 293 cells, drug selected,
and conditioned media was produced as described above.
The resulting secreted murine OPG 1-185.Fc fusion
product was purified by Protein-A column chromatography
(Pierce) using the manufacturers recommended

15 procedures.
      Murine OPG DNA encoding amino acid residues 1-194
fused to the Fc region of human IgG1 (muOPG Ct(194).Fc)
was constructed as follows. Mouse OPG cDNA from plasmid
pRcCMV Mu-Osteoprotegerin was amplified using the

20 following primer pairs:
      1333-82:
    5'-TCC CTT GCC CTG ACC ACT CTT-3'
    (SEQ ID NO:34)
      1333-81:

25 5'-CCT CTG CGG CCG CCT TTT GCG TGG CTT CTC TGT T-3'
    (SEQ ID NO:35)
      This primer pair amplifies the murine OPG cDNA
region encoding amino acid residues 70-194
(corresponding to bp 298-672) of the OPG reading frame.

30 The 3' primer contains a Not I restriction site which
is compatible with the in-frame Not I site of the Fc
fusion vector pFcA3. The product also spans a unique
EcoRI restriction site located at bp 436. The amplified
PCR product was cloned into the murine OPG[1-401] Fc

35 fusion vector as described above. The resulting pCEP4-
based expression vector encodes OPG residues 1-194

directly followed by all 227 amino acid residues of the
human IgG1 Fc region. The murine OPG 1-194.Fc fusion
vector was transfected into 293 cells, drug selected,
and conditioned media was produced. The resulting
5   secreted fusion product was purified by Protein-A
column chromatography (Pierce) using the manufacturers
recommended procedures.

Human OPG DNA encoding amino acids 1-401 fused to
the Fc region of human IgG1 was constructed as follows.
10   Human OPG DNA in plasmid pRcCMV-hu osteoprotegerin
(described in Example 5) was amplified using the
following oligonucleotide primers:
1254-90:
5'CCT CTG AGC TCA AGC TTG GTT TCC GGG GAC CAC AAT G-3'
15   (SEQ ID NO:36)
1254-95:
5'-CCT CTG CGG CCG CTA AGC AGC TTA TTT TTA CTG AAT GG-3'
(SEQ ID NO:37)

The resulting PCR product encodes the full-length
20   human OPG and creates a Not I restriction site which is
compatible with the in-frame Not I site Fc fusion
vector FcA3. The PCR product was directionally cloned
into the plasmid vector pCEP4 as described above. The
resulting expression vector encodes human OPG residues
25   1-401 directly followed by 227 amino acid residues of
the human IgG1 Fc region. Conditioned media from
transfected and drug selected cells was produced and
the huOPG Fl.Fc fusion product was purified by Protein-
A column chromatography (Pierce) using the
30   manufacturers recommended procedures.

Human OPG DNA encoding amino acid residues 1-201
fused to the Fc region of human IgG1 [huOPG Ct(201).Fc]
was constructed as follows. The cloned human OPG cDNA
from plasmid pRrCMV-hu osteoprotegerin was amplified by
35   PCR using the following oligonucleotide primer pair:
1254-90:

5'-CCT CTG AGC TCA AGC TTG GTT TCC GGG GAC CAC AAT G-3'
(SEQ ID NO:38)
    1254-92:
5'-CCT CTG CGG CCG CCA GGG TAA CAT CTA TTC CAC-3'
5 (SEQ ID NO:39)

    This primer pair amplifies the human OPG cDNA
region encoding amino acid residues 1-201 of the OPG
reading frame, and creates a Not I restriction site at
the 3' end which is compatable with the in-frame Not I
10 site Fc fusion vector FcA3. This product, when linked
to the Fc portion, encodes OPG residues 1-201 directly
followed by all 221 amino acid residues of the human
IgG1 Fc region. The PCR product was directionally
cloned into the plasmid vector pCEP4 as described
15 above. Conditioned media from transfected and drug
selected cells was produced, and the hu OPG Ct(201).Fc
fusion products purified by Protein-A column
chromatography (Pierce) using the manufacturer's
recommended procedures.
20   The following procedures were used to construct
and express unfused mouse and human OPG.

    A plasmid for mammalian expression of full-length
murine OPG (residues 1-401) was generated by PCR
amplification of the murine OPG cDNA insert from pRcCMV
25 Mu-Osteoprotegerin and subcloned into the expression
vector pDSRα (DeClerck et. atl. J. Biol. Chem. 266,
3893 (1991)). The following oligonucleotide primers
were used:
    1295-26:
30 5'-CCG AAG CTT CCA CCA TGA ACA AGT GGC TGT GCT GC-3'
(SEQ ID NO:40)
    1295-27:
5'-CCT CTG TCG ACT ATT ATA AGC AGC TTA TTT TCA CGG ATT G-3'
(SEQ ID NO:41)
35   The murine OPG full length reading frame was
amplified by PCR as described above. The PCR product

was purified and digested with restriction
endonucleases Hind III and XbaI (Boehringer Mannheim,
Indianapolis, IN) under the manufacturers recommended
conditions, then ligated to Hind III and Xba I digested
5   pDSRα. Recombinant clones were detected by restriction
endonuclease digestion, then sequenced to ensure no
mutations were produced during the PCR amplification
steps.

The resulting plasmid, pDSRα-muOPG was introduced
10   into Chinese hamster ovary (CHO) cells by calcium
mediated transfection (Wigler et al. (1977), Cell 11:
233). Individual colonies were selected based upon
expression of the dihydrofolate reductase (DHFR) gene
in the plasmid vector and several clones were isolated.
15   Expression of the murine OPG recombinant protein was
monitored by western blot analysis of CHO cell
conditioned media. High expressing cells were selected,
and OPG expression was further amplified by treatment
with methotrexate as described (DeClerck et al.,
20   ibid.). Conditioned media from CHO cell lines was
produced for further purification of recombinant
secreted murine OPG.

A plasmid for mammalian expression of full-length
human OPG (amino acids 1-401) was generated by
25   subcloning the cDNA insert in pRcCMV-hu Osteoprotegerin
directly into vector pDSRα (DeClerck et al., ibid). The
pRcCMV-OPG plasmid was digested to completion with Not
I, blunt ended with Klenow, then digested to completion
with XbaI. Vector DNA was digested with HindIII, blunt
30   ended with Klenow, then digested with XbaI, then
ligated to the OPG insert. Recombinant plasmids were
then sequenced to confirm proper orientation of the
human OPG cDNA.

The resulting plasmid pDSRα-huOPG was introduced
35   into Chinese hamster ovary (CHO) cells as described

above. Individual colonies were selected based upon
expression of the dihydrofolate reductase (DHFR) gene
in the plasmid vector and several clones were isolated.
Expression of the human OPG recombinant protein was
5   monitored by western blot analysis of CHO cell
conditioned media. High expressing clones were
selected, and OPG expression was further amplified by
treatment with methotrexate. Conditioned media from CHO
cell lines expressing human OPG was produced for
10   protein purification.

Expression vectors for murine OPG encoding
residues 1-185 were constructed as follows. Murine OPG
cDNA from pRcCMV-Mu OPG was amplified using the
following oligonucleotide primers:
15   1333-82:
5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:42)
1356-12:
5'-CCT CTG TCG ACT TAA CAC ACG TTG TCA TGT GTT GC-3'
(SEQ ID NO:43)
20   This primer pair amplifies the murine OPG cDNA
region encoding amino acids 63-185 of the OPG reading
frame (bp 278-645) and contains an artificial stop
codon directly after the cysteine codon (C185), which
is followed by an artificial Sal I restriction
25   endonuclease site. The predicted product contains an
internal Eco RI restriction site useful for subcloning
into a pre-existing vector. After PCR amplification,
the resulting purified product was cleaved with Eco RI
and Sal I restriction endonucleases, and the large
30   fragment was gel purified. The purified product was
then subcloned into the large restriction fragment of
an Eco RI and Sal I digest of pBluescript-muOPG Fl.Fc
described above. The resulting plasmid was digested
with Hind III and Xho I and the small fragment was gel
35   purified. This fragment, which contains a open reading
frame encoding residues 1-185 was then subcloned into a

Hind III and Xho I digest of the expression vector
pCEP4. The resulting vector, pmuOPG [1-185], encodes a
truncated OPG polypeptide which terminates at a
cysteine residue located at position 185. Conditioned
5   media from transfected and drug selected cells was
produced as described above.
        1333-82:
5'-TCC CTT GCC CTG ACC ACT CTT-3' (SEQ ID NO:44)
        1356-13:
10 5'-CCT CTG TCG ACT TAC TTT TGC GTG GCT TCT CTG TT-3'
(SEQ ID NO:45)
        This primer pair amplifies the murine OPG cDNA
region encoding amino acids 70-194 of the OPG reading
frame (bp 298-672) and contains an artificial stop
15 codon directly after the lysine codon (K194), which is
followed by an artificial Sal I restriction
endonuclease site. The predicted product contains an
internal Eco RI restriction site useful for subcloning
into a pre-existing vector. After PCR amplification,
20 the resulting purified product was cleaved with Eco RI
and Sal I restriction endonucleases, and the large
fragment was gel purified. The purified product was
then subcloned into the large restriction fragment of
an Eco RI and Sal I digest of pBluescript-muOPG Fl.Fc
25 described above. The resulting plasmid was digested
with Hind III and Xho I and the small fragment was gel
purified. This fragment, which contains a open reading
frame encoding residues 1-185 was then subcloned into a
Hind III and Xho I digest of the expression vector
30 pCEP4. The resulting vector, pmuOPG [1-185], encodes a
truncated OPG polypeptide which terminates at a lysine
at position 194. Conditioned media from transfected and
drug selected cells was produced as described above.
        Several mutations were generated at the 5' end of
35 the huOPG [22-401]-Fc gene that introduce either amino
acid substitutions, or deletions, of OPG between

                             101

residues 22 through 32. All mutations were generated
with the "QuickChange" Site-Directed Mutagenesis Kit"
(Stratagene, San Diego, CA) using the manfacturer's
recommended conditions. Briefly, reaction mix
5   containing huOPG [22-401]-Fc plasmid DNA template and
mutagenic primers were treated with Pfu polymerase in
the presence of deoxynucleotides, then amplified in a
thermocycler as described above. An aliqout of the
reaction is then transfected into competent E. coli
10   XL1-Blue by heatshock, then plated. Plasmid DNA from
transformants was then sequenced to verify mutations.
        The following primer pairs were used to delete
residues 22-26 of the human OPG gene, resulting in the
production of a huOPG [27-401]-Fc fusion protein:
15   1436-11:
     5'-TGG ACC ACC CAG AAG TAC CTT CAT TAT GAC-3' (SEQ ID NO:140)
          1436-12:
     5'-GTC ATA ATG AAG GTA CTT CTG GGT GGT CCA-3' (SEQ ID NO:141)
        The following primer pairs were used to delete
20   residues 22-28 of the human OPG gene, resulting in the
production of a huOPG [29-401]-Fc fusion protein:
          1436-17:
     5'-GGA CCA CCC AGC TTC ATT ATG ACG AAG AAA C-3' (SEQ ID NO:142)
          1436-18:
25   5'-GTT TCT TCG TCA TAA TGA AGC TGG GTG GTC C-3' (SEQ ID NO:143)
        The following primer pairs were used to delete
residues 22-31 of the human OPG gene, resulting in the
production of a huOPG [32-401]-Fc fusion protein:
          1436-27:
30   5'-GTG GAC CAC CCA GGA CGA AGA AAC CTC TC-3' (SEQ ID NO:144)
          1436-28:
     5'-GAG AGG TTT CTT CGT CCT GGG TGG TCC AC-3' (SEQ ID NO:145)
        The following primer pairs were used to change the
codon for tyrosine residue 28 to phenylalanine of the
35   human OPG gene, resulting in the production of a huOPG
[22-401]-Fc Y28F fusion protein:

1436-29:

5'-CGT TTC CTC CAA AGT TCC TTC ATT ATG AC-3' (SEQ ID NO:146)

1436-30:

5'-GTC ATA ATG AAG GAA CTT TGG AGG AAA CG-3' (SEQ ID NO:147)

5   The following primer pairs were used to change the
codon for proline residue 26 to alanine of the human
OPG gene, resulting in the production of a huOPG [22-
401]-Fc P26A fusion protein:

1429-83:

10   5'-GGA AAC GTT TCC TGC AAA GTA CCT TCA TTA TG-3 (SEQ ID NO:148)

1429-84:

5'-CAT AAT GAA GGT ACT TTG CAG GAA ACG TTT CC-3'(SEQ ID NO:149)

Each resulting muOPG [22-401]-Fc plasmid
containing the appropriate mutation was then
15   transfected into human 293 cells, the mutant OPG-Fc
fusion protein purified from conditioned media as
described above. The biological activity of each
protein was assessed the in vitro osteoclast forming
assay described in Example 11.

20   **EXAMPLE 8**

                  Expression of OPG in E. coli

A. Bacterial Expression Vectors

     pAMG21

     The expression plasmid pAMG21 can be derived from
25   the Amgen expression vector pCFM1656 (ATCC #69576)
which in turn be derived from the Amgen expression
vector system described in US Patent No. 4,710,473. The
pCFM1656 plasmid can be derived from the described
pCFM836 plasmid (Patent No. 4,710,473) by: (a)
30   destroying the two endogenous NdeI restriction sites by
end filling with T4 polymerase enzyme followed by blunt
end ligation; (b) replacing the DNA sequence between
the unique AatII and ClaI restriction sites containing
the synthetic P_L promoter with a similar fragment
35   obtained from pCFM636 (patent No. 4,710,473) containing
the PL promoter

AatII
5'   CTAATTCCGCTCTCACCTACCAAACAATGCCCCCCTGCAAAAAATAAATTCATAT-

3'TGCAGATTAAGGCGAGAGTGGATGGTTTGTTACGGGGGGACGTTTTTTATTTAAGTATA-

5 -AAAAAACATACAGATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAA-

   -TTTTTTGTATGTCTATTGGTAGACGCCACTATTTAATAGAGACCGCCACAACTGTATTT-

   -TACCACTGGCGGTGATACTGAGCACAT 3' (SEQ ID NO:53)

   -ATGGTGACCGCCACTATGACTCGTGTAGC5' (SEQ ID NO:54)

10   ClaI

   and then (c) substituting the small DNA sequence

   between the unique ClaI and KpnI restriction sites with

   the following oligonucleotide:

   5'   CGATTTGATTCTAGAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGGTAC3'

15 (SEQ ID NO:48)

   3'   TAAACTAAGATCTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGC 5'

   (SEQ ID NO:49)

       ClaI   KpnI

       The expression plasmid pAMG21 can then be derived

20 from pCFM1656 by making a series of site directed base

   changes by PCR overlapping oligo mutagenesis and DNA

   sequence substitutions. Starting with the BglII site

   (plasmid bp # 180) immediately 5' to the plasmid

   replication promoter PcopB and proceeding toward the

25 plasmid replication genes, the base pair changes are as

   follows:

Table 4

pAMG21 bp #	bp in pCFM1656	bp changed to in pAMG21
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 679	G/C	T/A
# 980	T/A	C/G
# 994	G/C	A/T
# 1004	A/T	C/G
# 1007	C/G	T/A
# 1028	A/T	T/A
# 1047	C/G	T/A
# 1178	G/C	T/A
# 1466	G/C	T/A
# 2028	G/C	bp deletion
# 2187	C/G	T/A

# 2480	A/T	T/A
# 2499-2502	AGTG	GTCA
	TCAC	CAGT
# 2642	TCCGAGC	7 bp deletion
	AGGCTCG	
# 3435	G/C	A/T
# 3446	G/C	A/T
# 3643	A/T	T/A

The DNA sequence between the unique AatII
(position #4364 in pCFM1656) and SacII (position #4585
in pCFM1656) restriction sites is substituted with the
following DNA sequence:

```
[AatII sticky end]       5'   GCGTAACGTATGCATGGTCTCC-
(position #4358 in pAMG21)   3' TGCACGCATTGCATACGTACCAGAGG-

-CCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT-
-GGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAGCTTTCTGA-

-GGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGC-
-CCCGGAAAGCAAAATAGACAACAAACAGCCACTTGCGAGAGGACTCATCCTGTTTAGGCG-

-CGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGC-
-GCCCTCGCCTAAACTTGCAACGCTTCGTTGCCGGGCCTCCCACCGCCCGTCCTGCGGGCG-

-CATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGT-
-GTATTTGACGGTCCGTAGTTTAATTCGTCTTCCGGTAGGACTGCCTACCGGAAAAACGCA-

                        AatII
-TTCTACAAACTCTTTTGTTTATTTTTCTAAATACATTCAAATATGGACGTCGTACTTAAC-
-AAGATGTTTGAGAAAACAAATAAAAAGATTTATGTAAGTTTATACCTGCAGCATGAATTG-

-TTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAAATTGCTTTAGAAATACTTTGGCAGC-
-AAAATTTCATACCCGTTAGTTAACGAGGACAATTTTAACGAAATCTTTATGAAACCGTCG-

-GGTTTGTTGTATTGAGTTTCATTTGCGCATTGGTTAAATGGAAAGTGACCGTGCGCTTAC-
-CCAAACAACATAACTCAAAGTAAACGCGTAACCAATTTACCTTTCACTGGCACGCGAATG-

-TACAGCCTAATATTTTTGAAATATCCCAAGAGCTTTTTCCTTCGCATGCCCACGCTAAAC-
-ATGTCGGATTATAAAAACTTTATAGGGTTCTCGAAAAAGGAAGCGTACGGGTGCGATTTG-

-APTCTTTTTCTCTTTTGGTTAAATCGTTGTTTGATTTATTATTTGCTATATTTATTTTTC-
-TAAGAAAAAGAGAAAACCAATTTAGCAACAAACTAAATAATAAACGATATAAATAAAAAG-

-GATAATTATCAACTAGAGAAGGAACAATTAATGGTATAGTGTTCATACACGCATGTAAAAATA-
-CTATTAATAAGTTGATCTCTTCCTTGTTAATTACCATACAAGTATGTGCGTACATTTTTAT-

-AACTATCTATATAGTTGTCTTTCTCTGAATGTGCAAAACTAAGCATTCCGAAGCCATTAT-
-TTGATAGATATATCAACAGAAAGAGACTTACACGTTTTGATTCGTAAGGCTTCGGTAATA-

-TAGCAGTATGAATAGGGAAACTAAACCCAGTGATAAGACCTGATGATTTCGCTTCTTTAA-
-ATCGTCATACTTATCCCTTTGATTTGGGTCACTATTCTGGACTACTAAAGCGAAGAAATT-

-TTACATTTGGAGATTTTTTATTTACAGCATTGTTTTCAAAATATATTCCAATTAATCGGTG-
-AATGTAAACCTCTAAAAAATAAATGTCGTAACAAAAGTTTATATAAGGTTAATTAGCCAC-

-AATGATTGGAGTTAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCAT-
```

```
-TTACTAACCTCAATCTTATTAGATGATATCCTAGTATAAAATAATTTAATCGCAGTAGTA-

-AATATTGCCTCCATTTTTTAGGGTAATTATCCAGAATTGAAATATCAGATTTAACCATAG-
-TTATAACGGAGGTAAAAAATCCCATTAATAGGTCTTAACTTTATAGTCTAAATTGGTATC-

-AATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTACCATTTTAGTCATATCAG-
-TTACTCCTATTTACTAGCGCTCATTTATTATAAGTGTTACATGGTAAAATCAGTATAGTC-

-ATAAGCATTGATTAATATCATTATTGCTTCTACAGGCTTTAATTTTATTAATTATTCTGT-
-TATTCGTAACTAATTATAGTAATAACGAAGATGTCCGAAATTAAAATAATTAATAAGACA-

-AAGTGTCGTCGGCATTTATGTCTTTCATACCCATCTCTTTATCCTTACCTATTGTTTGTC-
-TTCACAGCAGCCGTAAATACAGAAAGTATGGGTAGAGAAATAGGAATGGATAACAAACAG-

-GCAAGTTTTGCGTGTTATATATCATTAAAACGGTAATAGATTGACATTTGATTCTAATAA-
-CGTTCAAAACGCACAATATATAGTAATTTTGCCATTATCTAACTGTAAACTAAGATTATT-

-ATTGGATTTTTGTCACACTATTATATCGCTTGAAATACAATTGTTTAACATAAGTACCTG-
-TAACCTAAAAACAGTGTGATAATATAGCGAACTTTATGTTAACAAATTGTATTCATGGAC-

-TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGATTAATCGATTTGATT-
-ATCCTAGCATGTCCAAATGCGTTCTTTTACCAAACAATATCAGCTAATTAGCTAAACTAA-

-CTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGA-
-GATCTAAACAAAATTGATTAATTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGCT-
```
```
                            SacII
-GCTCACTAGTGTCGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAA-
-CGAGTGATCACAGCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTTCTT-

-GAAGAAGAAGAAGAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA-
-CTTCTTCTTCTTTCTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTAT-

-ACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGG-
-TGATCGTATTGGGGAACCCCGGAGATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCC-
```
```
-AACCGCTCTTCACGCTCTTCACGC 3' [SacII sticky end] (SEQ ID NO:50)
-TTGGCGAGAAGTGCGAGAAGTG   5' (position #5904 in pAMG21)
(SEQ ID NO:46)
```

During the ligation of the sticky ends of this
substitution DNA sequence, the outside AatII and SacII
sites are destroyed. There are unique AatII and SacII
sites in the substituted DNA.

pAMG22-His

The expression plasmid pAMG22-His can be derived
from the Amgen expression vector pAMG22 by substituting
the small DNA sequence between the unique NdeI (#4795)
and EcoRI (#4818) restriction sites of pAMG22 with the
following oligonucleotide duplex:

```
 NdeI              NheI         EcoRI
 5' TATGAAACATCATCACCATCACCATCATGCTAGCGTTAACGCGTTGG   3'

 (SEQ ID NO:51)

 3'  ACTTTGTAGTAGTGGTAGTGGTAGTACGATCGCAATTGCGCAACCTTAA 5'
```

(SEQ ID NO:52)
   MetLysHisHisHisHisHisHisAlaSerValAsnAlaLeuGlu
(SEQ ID NO:168)

pAMG22

5   The expression plasmid pAMG22 can be derived from
the Amgen expression vector pCFM1656 (ATCC #69576)
which in turn be derived from the Amgen expression
vector system described in US Patent No. 4,710,473
granted December 1, 1987. The pCFM1656 plasmid can be
10   derived from the described pCFM836 plasmid (Patent No.
4,710,473) by: (a) destroying the two endogenous NdeI
restriction sites by end filling with T4 polymerase
enzyme followed by blunt end ligation; (b) replacing
the DNA sequence between the unique AatII and ClaI
15   restriction sites containing the synthetic PL promoter
with a similar fragment obtained from pCFM636 (patent
No. 4,710,473) containing the PL promoter

AatII
5'   CTAATTCCGCTCTCACCTACCAAACAATGCCCCCCTGCAAAAAATAAATTCATAT-
20 3'TGCAGATTAAGGCGAGAGTGGATGGTTTGTTACGGGGGGACGTTTTTTATTTAAGTATA-

-AAAAAACATACAGATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAA-
-TTTTTTGTATGTCTATTGGTAGACGCCACTATTTAATAGAGACCGCCACAACTGTATTT-

25 -TACCACTGGCGGTGATACTGAGCACAT   3' (SEQ ID NO:53)
    -ATGGTGACCGCCACTATGACTCGTGTAGC5' (SEQ ID NO:54)
                       ClaI
    and then (c) substituting the small DNA sequence
    between the unique ClaI and KpnI restriction sites with
30   the following oligonucleotide:
    5' CGATTTGATTCTAGAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGGTAC   3'
    (SEQ ID NO:55)
    3'   TAAACTAAGATCTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGC 5'
    (SEQ ID NO:56)
35   ClaI   KpnI

The expression plasmid pAMG22 can then be derived from pCFM1656 by making a series of site directed base changes by PCR overlapping oligo mutagenesis and DNA sequence substitutions. Starting with the BglII site
5   (plasmid bp # 180) immediately 5' to the plasmid replication promoter PcopB and proceeding toward the plasmid replication genes, the base pair changes are as follows:

<div align="center">Table 5</div>

pAMG22 bp #	bp in pCFM1656	bp changed to in pAMG22
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 679	G/C	T/A
# 980	T/A	C/G
# 994	G/C	A/T
# 1004	A/T	C/G
# 1007	C/G	T/A
# 1028	A/T	T/A
# 1047	C/G	T/A
# 1178	G/C	T/A
# 1466	G/C	T/A
# 2028	G/C	bp deletion
# 2187	C/G	T/A
# 2480	A/T	T/A
# 2499-2502	AGTG TCAC	GTCA CAGT
# 2642	TCCGAGC AGGCTCG	7 bp deletion
# 3435	G/C	A/T
# 3446	G/C	A/T
# 3643	A/T	T/A

The DNA sequence between the unique AatII (position #4364 in pCFM1656) and SacII (position #4585 in pCFM1656) restriction sites is substituted with the following DNA sequence:

[AatII sticky end] (position #4358 in pAMG22)

5'   GCGTAACGTATGCATGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAA-
3' TGCACGCATTGCATACGTACCAGAGGGGTACGCTCTCATCCCTTGACGGTCCGTAGTT-

-ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTG-
5   -TATTTTGCTTTCCGAGTCAGCTTTCTGACCCGGAAAGCAAAATAGACAACAAACAGCCAC-

-AACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGG-
10   -TTGCGAGAGGACTCATCCTGTTTAGGCGGCCCTCGCCTAAACTTGCAACGGCTTCGTTGCC-

-CCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAG-
-GGGCCTCCCACCGCCCGTCCTGCGGGCGGTATTTGACGGTCCGTAGTTTAATTCGTCTTC-

-GCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTGTTTATTTTTCTAAAT-
15   -CGGTAGGACTGCCTACCGGAAAAACGCAAAGATGTTTGAGAAAACAAATAAAAAGATTTA-

            AatII
-ACATTCAAATATGGACGTCTCATAATTTTTAAAAAATTCATTTGACAAATGCTAAAATTC-
-TGTAAGTTTATACCTGCAGAGTATTAAAAATTTTTTAAGTAAACTGTTTACGATTTTAAG-
20
-TTGATTAATATTCTCAATTGTGAGCGCTCACAATTTATCGATTTGATTCTAGATTTGTTT-
-AACTAATTATAAGAGTTAACACTCGCGAGTGTTAAATAGCTAAACTAAGATCTAAACTCA-

-TAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGAGCTCACTAGTGT-
25   -ATTGATTAATTTCCTCCTTATTGTATACCAATTGCGCAACCTTAAGCTCGAGTGATCACA-

              SacII
-CGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAAGAAGAAGAAGAA-
-GCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTTCTTCTTCTTCTTCTT-
30
-GAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACC-
-CTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGG-

-CCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACCGCTCTTCA-
35   -GGAACCCCGGAGATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCCTTGGCGAGAAGT-

-CGCTCTTCACGC 3' (SEQ ID NO:58)
-GCGAGAAGTG 5' (SEQ ID NO:57)

40   [SacII sticky end] (position #5024 in pAMG22)
        During the ligation of the sticky ends of this

substitution DNA sequence, the outside AatII and SacII

sites are destroyed. There are unique AatII and SacII

sites in the substituted DNA.

45   B. Human OPG Met[32-401]

        In the example, the expression vector used was

pAMG21, a derivative of pCFM1656 (ATCC accession no.

69576) which contains appropriate restriction sites for

insertion of genes downstream from the lux PR promoter.

50   (See U.S. Patent No. 5,169,318 for description of the

lux expression system). The host cell used was GM120

(ATCC accession no. 55764). This host ·has the lacIQ

promoter and lacI gene integrated into a second site in

the host chromosome of a prototrophic E. coli K12 host.
Other commonly used E. coli expression vectors and host
cells are also suitable for expression.

A DNA sequence coding for an N-terminal methionine
5   and amino acids 32-401 of the human OPG polypeptide was
placed under control of the luxPR promoter in the
plasmid expression vector pAMG21 as follows. To
accomplish this, PCR using oligonucleotides #1257-20
and #1257-19 as primers was performed using as a
10   template plasmid pRcCMV-Hu OPG DNA containing the human
OPG cDNA and thermocycling for 30 cycles with each
cycle being: 94°C for 20 seconds, followed by 37°C for
30 seconds, followed by 72°C for 30 seconds. The
resulting PCR sample was resolved on an agarose gel,
15   the PCR product was excised, purified, and restricted
with KpnI and BamHI restriction endonucleases and
purified. Synthetic oligonucleotides #1257-21 and
#1257-22 were phophorylated individually using T4
polynucleotide kinase and ATP, and were then mixed
20   together, heated at 94°C and allowed to slow cool to
room temperature to form an oligonucleotide linker
duplex containing NdeI and KpnI sticky ends. The
phosphorylated linker duplex formed between
oligonucleotides #1257-21 and #1257-22 containing NdeI
25   and KpnI cohesive ends (see Figure 14A) and the KpnI
and BamHI digested and purified PCR product generated
using oligo primers #1257-20 and #1257-19 (see above)
was directionally inserted between two sites of the
plasmid vector pAMG21, namely the NdeI site and BamHI
30   site, using standard recombinant DNA methodology (see
Figure 14A and sequences below). The synthetic linker
utilized E. coli codons and provided for a N-terminal
methionine.

Two clones were selected and plasmid DNA isolated,
35   and the human OPG insert was subsequently DNA sequence
confirmed. The resulting pAMG21 plasmid containing

amino acids 32-401 of the human OPG polypeptide
immediately preceded in frame by a methionine is
referred to as pAMG21-huOPG met[32-401] or pAMG21-huOPG
met[32-401].

5   Oligo#1257-19:

5'-TACGCACTGGATCCTTATAAGCAGCTTATTTTTACTGATTGGAC-3'

(SEQ ID NO:59)

         Oligo#1257-20:

5'-GTCCTCCTGGTACCTACCTAAAACAAC-3' (SEQ ID NO:60)

10   Oligo#1257-21:

5'-TATGGATGAAGAAACTTCTCATCAGCTGCTGTGTGATAAATGTCCGCCGGGTAC   -3'

(SEQ ID NO:61)

         Oligo#1257-22:

5'-CCGGCGGACATTTATCACACAGCAGCTGATGAGAAGTTTCTTCATCCA-3'

15   (SEQ ID NO:47)

         Cultures of pAMG21-huOPG met[32-401] in E. coli
GM120 in 2XYT media containing 20 μg/ml kanamycin were
incubated at 30°C prior to induction. Induction of
huOPG met[32-401] gene product expression from the
20   luxPR promoter was achieved following the addition of
the synthetic autoinducer N-(3-oxohexanoyl)-DL-
homoserine lactone to the culture media to a final
concentration of 30 ng/ml and cultures were incubated
at either 30°C or 37°C for a further 6 hours. After 6
25   hours, the bacterial cultures were examined by
microscopy for the presence of inclusion bodies and
were then pelletted by centrifugation. Refractile
inclusion bodies were observed in induced cultures
indicating that some of the recombinant huOPG met[32-
30   401] gene product was produced insolubly in E. coli.
         Some bacterial pellets were resuspended in 10mM
Tris-HCl/pH8, 1mM EDTA and lysed directly by addition
of 2X Laemlli sample buffer to 1X final, and β-
mercaptoethanol to 5% final concentration, and analyzed
35   by SDS-PAGE. A substantially more intense coomassie
stained band of approximately 42kDa was observed on a

SDS-PAGE gel containing total cell lysates of 30°C and
37°C induced cultures versus lane 2 which is a total
cell lysate of a 30°C uninduced culture (Figure 14B).
The expected gene product would be 370 amino acids in
5   length and have an expected molecular weight of about
42.2 kDa.

        Following induction at 37 °C for 6 hours, an
additional culture was pelleted and either processed
for isolation of inclusion bodies (see below) or
10   processed by microfluidizing. The pellet processed for
microfluidizing was resuspended in 25mM Tris-HCl/pH8,
0.5M NaCl buffer and passed 20 times through a
Microfluidizer Model 1108 (Microfluidics Corp.) and
collected. An aliquot was removed of the collected
15   sample (microfluidized total lysate), and the remainder
was pelleted at 20,000 x g for 20 minutes. The
supernatant following centrifugation was removed
(microfluidized soluble fraction) and the pellet
resuspended in a 25mM Tris-HCl/pH8, 0.5M NaCl, 6M urea
20   solution (microfluidized insoluble fraction). To an
aliquot of either the total soluble, or insoluble
fraction was added to an equal volume of 2X Laemalli
sample buffer and β-mercaptoethanol to 5% final
concentration. The samples were then analyzed by
25   SDS-PAGE. A significant amount of recombinant huOPG
met[32-401] gene product appeared to be found in the
insoluble fraction.

        To purify the recombinant protein, inclusion
bodies were purified as follows: Bacterial cells were
30   separated from media by density gradient centrifugation
in a Beckman J-6B centrifuge equipped with a JS-4.2
rotor at 4,900 x g for 15 minutes at 4°C. The bacterial
pellet was resuspended in 5 ml of water and then
diluted to a final volume of 10 ml with water. This
35   suspension was transferred to a stainless steel cup

cooled in ice and subjected to sonic disruption using a
Branson Sonifier equipped with a standard tip (power
setting=5, duty cycle=95%, 80 bursts). The sonicated
cell suspension was centrifuged in a Beckman Optima TLX
5   ultracentrifuge equipped with a TLA 100.3 rotor at
195,000 x g for 5 to 10 minutes at 23°C. The
supernatant was discarded and the pellet rinsed with a
stream of water from a squirt bottle. The pellets were
collected by scraping with a micro spatula and
10   transferred to a glass homogenizer (15 ml capacity).
Five ml of Percoll solution (75% liquid Percoll, 0.15 M
sodium chloride) was added to the homogenizer and the
contents are homogenized until uniformly suspended. The
volume was increased to 19.5 ml by the addition of
15   Percoll solution, mixed, and distributed into 3 Beckman
Quick-Seal tubes (13 x 32 mm). Tubes were sealed
according to manufacturers instructions. The tubes were
spun in a Beckman TLA 100.3 rotor at 23°C, 20,000 rpm
(21,600 x g), 30 minutes. The tubes were examined for
20   the appropriate banding pattern. To recover the
refractile bodies, gradient fractions were recovered
and pooled, then diluted with water. The inclusion
bodies were pelleted by centrifugation, and the protein
concentration estimated following SDS-PAGE.
25   An aliquot of inclusion bodies isolated as
described below was dissolved into 1X Laemlli sample
buffer with 5% β-mercaptoethanol and resolved on a SDS-
PAGE gel and the isolated inclusion bodies provide a
highly purified recombinant huOPG[32-401] gene product.
30   The major ~42 kDa band observed after resolving
inclusion bodies on a SDS-polyacrylamide gel was
excised from a separate gel and the N-terminal amino
acid sequence determined essentially as described
(Matsudaira et al. J. Biol. Chem. 262, 10-35 (1987)).
35   The following sequence was determined after 19 cycles:

NH$_2$ -MDEETSHQLLCDKCPPGTY-COOH (SEQ ID NO:62)

This sequence was found to be identical to the first 19
amino acids encoded by the pAMG21 Hu-OPG met[32-401]
expression vector, produced by a methionine residue
5   provided by the bacterial expression vector.

C. Human OPG met[22-401]

A DNA sequence coding for an N-terminal methionine and amino acids 22 through 401 of human OPG was placed under control of the luxPR promoter in a prokaryotic
5   plasmid expression vector pAMG21 as follows. Isolated plasmid DNA of pAMG21-huOPG met[32-401] (see Section B) was cleaved with KpnI and BamHI restriction endonucleases and the resulting fragments were resolved on an agarose gel. The B fragment (about 1064 bp
10 fragment) was isolated from the gel using standard methodology. Synthetic oligonucleotides (oligos) #1267-06 and #1267-07 were phosphorylated individually and allowed to form an oligo linker duplex, which contained NdeI and KpnI cohesive ends, using methods described in
15 Section B. The synthetic linker duplex utilized E. coli codons and provided for an N-terminal methionine. The phosphorylated oligo linker containing NdeI and KpnI cohesive ends and the isolated about 1064 bp fragment of pAMG21-huOP met[32-401] digested with KpnI and BamHI
20 restriction endonucleases were directionally inserted between the NdeI and BamHI sites of pAMG21 using standard recombinant DNA methodology. The ligation mixture was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol.
25 Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the huOPG-met[22-401] gene.

Oligo #1267-06:

5'-TAT GGA AAC TTT TCC TCC AAA ATA TCT TCA TTA TGA TGA AGA AAC TTC
30 TCA TCA GCT GCT GTG TGA TAA ATG TCC GCC GGG TAC-3'

(SEQ ID NO:63)

Oligo #1267-07:

5'-CCG GCG GAC ATT TAT CAC ACA GCA GCT GAT GAG AAG TTT CTT CAT
CAT AAT GAA GAT ATT TTG GAG GAA AAG TTT CCA-3'
35 (SEQ ID NO:64)

Cultures of pAMG21-huOPG-met[22-401] in E. coli
host 393 were placed in 2XYT media containing 20 µg/ml
kanamycin and were incubated at 30°C prior to
induction. Induction of recombinant gene product

5   expression from the luxPR promoter of vector pAMG21 was
achieved following the addition of the synthetic
autoinducer N-(3-oxohexanoyl)-DL-homoserine lactone to
the culture media to a final concentration of 30 ng/ml
and incubation at either 30°C or 37°C for a further 6

10 hours. After 6 hours, bacterial cultures were pelleted
by centrifugation (=30°C I+6 or 37°C I+6). Bacterial
cultures were also either pelleted just prior to
induction (=30°C PreI) or alternatively no autoinducer
was added to a separate culture which was allowed to

15 incubate at 30°C for a further 6 hours to give an
uninduced (UI) culture (=30°C UI). Bacterial pellets of
either 30°C PreI, 30°C UI, 30°C I+6, or 37°C I+6
cultures were resuspended, lysed, and analyzed by SDS-
polyacrylamide gel electrophoresis (PAGE) as described

20 in Section B. Polyacrylamide gels were either stained
with coomassie blue and/or Western transferred to
nitrocellulose and immunoprobed with rabbit anti-mu
OPG-Fc polyclonal antibody as described in Example 10.
The level of gene product following induction compared

25 to either an uninduced (30°C UI) or pre-induction (30°C
PreI) sample.

D. Murine OPG met[22-401]

A DNA sequence coding for an N-terminal methionine
and amino acids 22 through 401 of the murine (mu) OPG

30 (OPG) polypeptide was placed under control of the luxPR
promoter in a prokaryotic plasmid expression vector
pAMG21 as follows. PCR was performed using
oligonucleotides #1257-16 and #1257-15 as primers,
plasmid pRcCMV-Mu OPG DNA as a template and

35 thermocycling conditions as described in Section B. The
PCR product was purified and cleaved with KpnI and

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BamHI restriction endonucleases as described in Section
B. Synthetic oligos #1260-61 and #1260-82 were
phosphorylated individually and allowed to form an
oligo linker duplex with NdeI and KpnI cohesive ends
5   using methods described in Section B. The synthetic
linker duplex utilized E. coli codons and provided for
an N-terminal methionine. The phosphorylated linker
duplex formed between oligos #1260-61 and #1260-82
containing NdeI and KpnI cohesive ends and the KpnI and
10 BamHI digested and purified PCR product generated using
oligo primers #1257-16 and #1257-15 were directionally
inserted between the NdeI and BamHI sites of pAMG21
using standard methodology. The ligation mixture was
transformed into E. coli host 393 by electroporation
15 utilizing the manufacturer's protocol. Clones were
selected, plasmid DNA was isolated, and DNA sequencing
was performed to verify the DNA sequence of the MuOPG
met[22-401] gene.

     Expression of recombinant muOPG met[22-401]
20 polypeptide from cultures of 393 cells harboring
plasmid pAMG21-MuOPG met[22-401] following induction
was determined using methods described in Section C.

     Oligo #1257-15:
5'-TAC GCA CTG GAT CCT TAT AAG CAG CTT ATT TTC ACG GAT TGA AC-3'
25 (SEQ ID NO:65)

     Oligo #1257-16:
5'-GTG CTC CTG GTA CCT ACC TAA AAC AGC ACT GCA CAG TG-3'
     (SEQ ID NO:66)

     Oligo #1260-61:
30 5'-TAT GGA AAC TCT GCC TCC AAA ATA CCT GCA TTA CGA TCC GGA AAC TGG
TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC GGG TAC-3'
     (SEQ ID NO:67)

     Oligo #1260-82:
5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT
35 AAT GCA GGT ATT TTG GAG GCA GAG TTT CCA-3'
     (SEQ ID NO:68)

E. Murine OPG met[32-401]

A DNA sequence coding for an N-terminal methionine
and amino acids 32 through 401 of murine OPG was placed
under control of the luxPR promoter in a prokaryotic

5   plasmid expression vector pAMG21 as follows. To
accomplish this, Synthetic oligos #1267-08 and #1267-09
were phosphorylated individually and allowed to form an
oligo linker duplex using methods described in Section
B. The synthetic linker duplex utilized E. coli codons

10   and provided for an N-terminal methionine. The
phosphorylated linker duplex formed between oligos
#1267-08 and #1267-09 containing NdeI and KpnI cohesive
ends, and the KpnI and BamHI digested and purified PCR
product described earlier (see Section D), was

15   directionally inserted between the NdeI and BamHI sites
of pAMG21 using standard methodology. The ligation
mixture was transformed into E. coli host 393 by
electroporation utilizing the manufacturer's protocol.
Clones were selected, plasmid DNA was isolated, and DNA

20   sequencing was performed to verify the DNA sequence of
the muOPG-met[32-401] gene.

      Expression of recombinant muOPG-met [32-401]
polypeptide from cultures of 393 cells harboring the
pAMG21 recombinant plasmid following induction was

25   determined using methods described in Section C.

      Oligo #1267-08:
5'-TAT GGA CCC AGA AAC TGG TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC
GGG TAC-3' (SEQ ID NO:69)
      Oligo #1267-09:

30   5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CTG GGT
CCA-3' (SEQ ID NO:70)
      F. Murine OPG met-lys[22-401]

      A DNA sequence coding for an N-terminal methionine
followed by a lysine residue and amino acids 22 through

35   401 of murine OPG was placed under control of the lux
PR promoter in prokaryotic expression vector pAMG21 as

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follows. Synthetic oligos #1282-95 and #1282-96 were
phosphorylated individually and allowed to form an
oligo linker duplex using methods described in Section
B. The synthetic linker duplex utilized E. coli codons

5   and provided for an N-terminal methionine. The
phosphorylated linker duplex formed between oligos
#1282-95 and #1282-96 containing NdeI and KpnI cohesive
ends and the KpnI and BamHI digested and purified PCR
product described in Section D was directionally

10 inserted between the NdeI and BamHI sites in pAMG21
using standard methodology. The ligation mixture was
transformed into E. coli host 393 by electroporation
utilizing the manufacturer's protocol. Clones were
selected, plasmid DNA was isolated, and DNA sequencing

15 was performed to verify the DNA sequence of the MuOPG-
Met-Lys[22-401] gene.

        Expression of recombinant MuOPG Met-Lys[22-401]
polypeptide from transformed 393 cells harboring the
recombinant pAMG21 plasmid following induction was

20 determined using methods described in Section C.

        Oligo #1282-95:
5'-TAT GAA AGA AAC TCT GCC TCC AAA ATA CCT GCA TTA CGA TCC GGA AAC
TGG TCA TCA GCT GCT GTG TGA TAA ATG TGC TCC GGG TAC-3'
(SEQ ID NO:71)

25   Oligo #1282-96:
5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT
AAT GCA GGT ATT TTG GAG GCA GAG TTT CTT TCA-3' (SEQ ID NO:72)
G. Murine OPG met-lys-(his)7[22-401]

        A DNA sequence coding for N-terminal residues Met-

30 Lys-His-His-His-His-His-His (=MKH) followed by
amino acids 22 through 401 of Murine OPG was placed
under control of the lux PR promoter in prokaryotic
expression vector pAMG21 as follows. PCR was performed
using oligonucleotides #1300-50 and #1257-15 as primers

35 and plasmid pAMG21-muOPG-met[22-401] DNA as template.
Thermocycling conditions were as described in Section

B. The resulting PCR sample was resolved on an agarose
gel, the PCR product was excised, purified, cleaved
with NdeI and BamHI restriction endonucleases and
purified. The NdeI and BamHI digested and purified PCR
5   product generated using oligo primers #1300-50 and
#1257-15 was directionally inserted between the NdeI
and BamHI sites of pAMG21 using standard DNA
methodology. The ligation mixture was transformed into
E. coli host 393 by electroporation utilizing the
10   manufacturer's protocol. Clones were selected, plasmid
DNA was isolated, and DNA sequencing performed to
verify the DNA sequence of the muOPG-MKH[22-401] gene.
       Expression of recombinant MuOPG-MKH[22-401]
polypeptide from transformed 393 cultures harboring the
15   recombinant pAMG21 plasmid following induction was
determined using methods described in Section C.
       Oligo #1300-50:
5'-GTT CTC CTC ATA TGA AAC ATC ATC ACC ATC ACC ATC ATG AAA CTC TGC
CTC CAA AAT ACC TGC ATT ACG AT-3' (SEQ ID NO:73)
20   Oligo #1257-15: see Section D
H. Murine OPG met-lys[22-401](his)7
       A DNA sequence coding for a N-terminal met-lys,
amino acids 22 through 401 murine OPG, and seven
histidine residues following amino acid 401 (=muOPG
25   MK[22-401]-H7), was placed under control of the lux PR
promoter in prokaryotic expression vector pAMG21 as
follows. PCR was performed using oligonucleotides
#1300-49 and #1300-51 as primers and pAMG21-muOPG
met[22-401] DNA as template. Thermocycling conditions
30   were as described in Section B. The resulting PCR
sample was resolved on an agarose gel, the PCR product
was excised, purified, restricted with NdeI and BamHI
restriction endonucleases, and purified. The NdeI and
BamHI digested and purified.PCR product was
35   directionally inserted between the NdeI and BamHI sites
in pAMG21 using standard methodology. The ligation was

transformed into E. coli host 393 by electroporation
utilizing the manufacturer's protocol. Clones were
selected, plasmid DNA was isolated, and DNA sequencing
was performed to verify the DNA sequence of the muOPG
5   MK[22-401]-H7 gene.

    Expression of the recombinant muOPG MK-[22-401]-H7
polypeptide from a transformed 393 cells harboring the
recombinant pAMG21 plasmid following induction was
determined using methods described in Section C.

10   Oligo #1300-49:
5'-GTT CTC CTC ATA TGA AAG AAA CTC TGC CTC CAA AAT ACC TGC A-3'
(SEQ ID NO:74)

    Oligo #1300-51:
5'-TAC GCA CTG GAT CCT TAA TGA TGG TGA TGG TGA TGA TGT AAG CAG CTT
15 ATT TTC ACG GAT TGA ACC TGA TTC CCT A-3' (SEQ ID NO:75)

I. Murine OPG met[27-401]

    A DNA sequence coding for a N-terminal methionine
and amino acids 27 through 401 of murine OPG was placed
under control of the lux PR promoter of prokaryotic
20 expression vector pAMG21 as follows. PCR was performed
with oligonucleotides #1309-74 and #1257-15 as primers
and plasmid pAMG21-muOPG-met[22-401] DNA as template.
Thermocycling conditions were as described in Section
B. The resulting PCR sample was resolved on an agarose
25 gel, the PCR product was excised, purified, cleaved
with NdeI and BamHI restriction endonucleases, and
purified. The NdeI and BamHI digested and purified PCR
product was directionally inserted between the NdeI and
BamHI sites of pAMG21 using standard methodology. The
30 ligation mixture was transformed into E. coli host 393
by electroporation utilizing the manufacturer's
protocol. Clones were selected, plasmid DNA was
isolated, and DNA sequencing was performed to verify
the DNA sequence of the muOPG-met[27-401] gene.

35   Expression of recombinant muOPG-met[27-401]
polypeptide from a transfected 393 culture harboring

the recombinant pAMG21 plasmid following induction was
determined using methods described in Section C.

Oligo#1309-74:

5'-GTT CTC CTC ATA TGA AAT ACC TGC ATT ACG ATC CGG AAA CTG GTC AT-

5   3' (SEQ ID NO:76)

Oligo#1257-15: See Section D

J. Human OPG met[27-401]

A DNA sequence coding for a N-terminal methionine
and amino acids 27 through 401 of human OPG was placed
10 under control of the lux PR promoter of prokaryotic
expression vector pAMG21 as follows. PCR was performed
using oligonucleotides #1309-75 and #1309-76 as primers
and plasmid pAMG21-huOPG-met[22-401] DNA as template.
Thermocycling conditions were as described in Section
15 B. The resulting PCR sample was resolved on an agarose
gel, the PCR product was excised, purified, restricted
with AseI and BamHI restriction endonucleases, and
purified. The AseI and BamHI digested and purified PCR
product above was directionally inserted between the
20 NdeI and BamHI sites of pAMG21 using standard
methodology. The ligation mixture was transformed into
E. coli host 393 by electroporation utilizing the
manufacturer's protocol. Clones were selected, plasmid
DNA was isolated, and DNA sequencing was performed to
25 verify the DNA sequence of the huOPG-met[27-401] gene.

Expression of the recombinant huOPG-met[27-401]
polypeptide following induction of from transfected 393
cells harboring the recombinant pAMG21 plasmid was
determined using methods described in Section C.

30 Oligo #1309-75:

5'-GTT CTC CTA TTA ATG AAA TAT CTT CAT TAT GAT GAA GAA ACT T-3'
(SEQ ID NO:77)

Oligo #1309-76:

5'-TAC GCA CTG GAT CCT TAT AAG CAG CTT ATT TTT ACT GAT T-3'

35 (SEQ ID NO:78)

K. Murine OPG met[22-180]

A DNA sequence coding for a N-terminal methionine and amino acids 22 through 180 of murine OPG was placed under control of the lux PR promoter of prokaryotic

5   expression vector pAMG21 as follows. PCR was performed with oligonucleotides #1309-72 and #1309-73 as primers and plasmid pAMG21-muOPG-met[22-401] DNA as template. Thermocycling conditions were as described in Section B. The resulting PCR sample was resolved on an agarose

10 gel, the PCR product was excised, purified, restricted with NdeI and BamHI restriction endonucleases, and purified. The NdeI and BamHI digested and purified PCR product above was directionally inserted between the NdeI and BamHI sites of pAMG21 using standard

15 methodology. The ligation was transformed into E. coli host 393 by electroporation utilizing the manufacturer's protocol. Clones were selected, plasmid DNA was isolated, and DNA sequencing was performed to verify the DNA sequence of the muOPG-met[22-180] gene.

20   Expression of recombinant muOPG-met[22-180] polypeptide from transformed 393 cultures harboring the recombinant pAMG21 plasmid following induction was determined using methods described in Section C.

        Oligo #1309-72:

25 5'-GTT CTC CTC ATA TGG AAA CTC TGC CTC CAA AAT ACC TGC A-3'
    (SEQ ID NO:79)

        Oligo #1309-73:

    5'-TAC GCA CTG GAT CCT TAT GTT GCA TTT CCT TTC TGA ATT AGC A-3'
    (SEQ ID NO:80)

30 ### L. Murine OPG met[27-180]

        A DNA sequence coding for a N-terminal methionine and amino acids 27 through 180 of murine OPG was placed under the control of the lux PR promoter of prokaryotic expression vector pAMG21 as follows. PCR was performed

35 using oligonucleotides #1309-74 (see Section I) and #1309-73 (see Section K) as primers and plasmid pAMG21-

muOPG met[22-401] DNA as template. Thermocycling
conditions were as described in Section B. The
resulting PCR sample was resolved on an agarose gel,
the PCR product excised, purified, restricted with NdeI
5   and BamHI restriction endonucleases, and purified. The
NdeI and BamHI digested and purified PCR product above
was directionally inserted between the NdeI and BamHI
sites in pAMG21 using standard methodology. The
ligation mixture was transformed into E. coli host 393
10 by electroporation utilizing the manufacturer's
protocol. Clones were selected, plasmid DNA was
isolated, and DNA sequencing was performed to verify
the DNA sequence of the muOPG met[27-180] gene.
      Expression of recombinant muOPG met[27-180]
15 polypeptide from cultures of transformed 393 cells
harboring the recombinant pAMG21 plasmid following
induction was determined using methods described in
Section C.
M. Murine OPG met[22-189] and met[22-194]
20   A DNA sequence coding for a N-terminal methionine
and either amino acids 22 through 189, or 22 through
194 of murine OPG was placed under control of the lux
PR promoter of prokaryotic expression vector pAMG21 as
follows. The pair of synthetic oligonucleotides #1337-
25 92 and #1337-93 (=muOPG-189 linker) or #1333-57 and
#1333-58 (=muOPG-194 linker) were phosphorylated
individually and allowed to form an oligo linker duplex
pair using methods described in Section B. Purified
plasmid DNA of pAMG21-muOPG-met[22-401] was cleaved
30 with KpnI and BspEI restriction endonucleases and the
resulting DNA fragments were resolved on an agarose
gel. The ~413 bp B fragment was isolated using standard
recombinant DNA methodology. The phosphorylated oligo
linker duplexes formed between either oligos #1337-92
35 and #1337-93 (muOPG-189 linker) or oligos #1333-57 and
#1333-58 (muOPG-194 linker) containing BspEI and BamHI

cohesive ends, and the isolated ~413 bp B fragment of
plasmid pAMG21-muOPG-met[22-401] digested with KpnI and
BspEI restriction endonucleases above, was
directionally inserted between the KpnI and BamHI sites
5   of pAMG21-muOPG met[22-401] using standard methodology.
Each ligation mixture was transformed into E. coli host
393 by electroporation utilizing the manufacturer's
protocol. Clones were selected, plasmid DNA was
isolated, and DNA sequencing was performed to verify
10   the DNA sequence of either the muOPG-met[22-189] or
muOPG-met[22-194] genes.

     Expression of recombinant muOPG-met[22-189] and
muOPG-met[22-194] polypeptides from recombinant pAMG21
plasmids transformed into 393 cells was determined
15   using methods described in Section C.

     Oligo #1337-92:
5'-CCG GAA ACA GAT AAT GAG-3' (SEQ ID NO:81)
     Oligo #1337-93:
5'-GAT CCT CAT TAT CTG TTT-3' (SEQ ID NO:82)
20   Oligo #1333-57:
5'-CCG GAA ACA GAG AAG CCA CGC AAA AGT AAG-3'
(SEQ ID NO:83)
     Oligo #1333-58:
5'-GAT CCT TAC TTT TGC GTG GCT TCT CTG TTT-3'
25   (SEQ ID NO:84)

N. Murine OPG met[27-189] and met[27-194]

     A DNA sequence coding for a N-terminal methionine
and either amino acids 27 through 189, or 27 through
194 of murine OPG was placed under control of the lux
30   PR promoter of prokaryotic expression vector pAMG21 as
follows. Phosphorylated oligo linkers either "muOPG-189
linker" or "muOPG-194 linker" (see Section M)
containing BspEI and BamHI cohesive ends, and the
isolated ~413 bp B fragment of plasmid pAMG21-muOPG-
35   met[22-401] digested with KpnI and BspEI restriction
endonucleases were directionally inserted between the

KpnI and BamHI sites of plasmid pAMG21-muOPG-met[27-
401] using standard methodology. Each ligation was
transformed into E. coli host 393 by electroporation
utilizing the manufacturer's protocol. Clones were
5   selected, plasmid DNA was isolated, and DNA sequencing
was performed to verify the DNA sequence of either the
muOPG met[27-189] or muOPG met[27-194] genes.

Expression of recombinant muOPG met[27-189] and
muOPG met[27-194] following induction of 393 cells
10 harboring recombinant pAMG21 plasmids was determined
using methods described in Section C.

O. Human OPG met[22-185], met[22-189], met[22-194]

A DNA sequence coding for a N-terminal methionine
and either amino acids 22 through 185, 22 through 189,
15 or 22 through 194 of the human OPG polypeptide was
placed under control of the lux PR promoter of
prokaryotic expression vector pAMG21 as follows. The
pair of synthetic oligonucleotides #1331-87 and #1331-
88 (=huOPG-185 linker), #1331-89 and #1331-90 (=huOPG-
20 189 linker), or #1331-91 & #1331-92 (=huOPG-194 linker)
were phosphorylated individually and each allowed to
form an oligo linker duplex pair using methods
described in Section B. Purified plasmid DNA of pAMG21-
huOPG-met[27-401] was restricted with KpnI and NdeI
25 restriction endonucleases and the resulting DNA
fragments were resolved on an agarose gel. The ~407 bp
B fragment was isolated using standard recombinant DNA
methodology. The phophorylated oligo linker duplexes
formed between either oligos #1331-87 and #1331-88
30 (huOPG-185 linker), oligos #1331-89 and #1331-90
(huOPG-189 linker), or oligos #1331-91 and #1331-92
(huOPG-194 linker)[each linker contains NdeI and BamHI
cohesive ends], and the isolated ~407 bp B fragment of
plasmid pAMG21-huOPG-met[27-401] digested with KpnI and
35 NdeI restriction endonucleases above, was directionally
inserted between the KpnI and BamHI sites of plasmid

pAMG21-huOPG-met[22-401] using standard methodology.
Each ligation was transformed into E. coli host 393 by
electroporation utilizing the manufacturer's protocol.
Clones were selected, plasmid DNA was isolated, and DNA
5   sequencing was performed to verify the DNA sequence of
either the huOPG-met[22-185], huOPG-met[22-189], or
huOPG-met[22-194] genes.

    Expression of recombinant huOPG-met[22-185],
huOPG-met[22-189] or huOPG-met[22-194] in transformed
10 393 cells harboring recombinant pAMG21 plasmids
following induction was determined using methods
described in Section C.
        Oligo #1331-87:
    5'-TAT GTT AAT GAG-3' (SEQ ID NO:85)
15   Oligo #1331-88:
    5'-GAT CCT CAT TAA CA-3' (SEQ ID NO:86)
        Oligo #1331-89:
    5'-TAT GTT CCG GAA ACA GTT AAG-3' (SEQ ID NO:87)
        Oligo #1331-90:
20 5'-GAT CCT TAA CTG TTT CCG GAA CA-3' (SEQ ID NO:88)
        Oligo #1331-91:
    5'-TAT GTT CCG GAA ACA GTG AAT CAA CTC AAA AAT AAG-3'
    (SEQ ID NO:89)
        Oligo #1331-92:
25 5'-GAT CCT TAT TTT TGA GTT GAT TCA CTG TTT CCG GAA CA-3'
    (SEQ ID NO:90)
    P. Human OPG met[27-185], met[27-189], met [27-194]
        A DNA sequence coding for a N-terminal methionine
and either amino acids 27 through 185, 27 through 189,
30 or 27 through 194 of the human OPG polypeptide was
placed under control of the lux PR promoter of
prokaryotic expression vector pAMG21 as follows.
Phosphorylated oligo linkers "huOPG-185 linker",
"huOPG-189 linker", or "huOPG-194 linker" (See Section
35 O) each containing NdeI and BamHI cohesive ends, and
the isolated ~407 bp B fragment of plasmid pAMG21-

huOPG-met[27-401] digested with KpnI and NdeI
restriction endonucleases (See Section O) were
directionally inserted between the KpnI and BamHI sites
of plasmid pAMG21-huOPG-met[27-401] (See Section J)
using standard methodology. Each ligation was
transformed into E. coli host 393 by electroporation
utilizing the manufacturer's protocol. Clones were
selected, plasmid DNA isolated, and DNA sequencing
performed to verify the DNA sequence of either the
huOPG-met[27-185], huOPG-met[27-189], or huOPG-met[27-
194] genes.

     Expression of recombinant huOPG-met[27-185],
huOPG-met[27-189], and huOPG-met[27-194] from
recombinant pAMG21 plasmids transformed into 393 cells
was determined using methods described in Section C.
O. Murine OPG met[27-401] (P33E, G36S, A45P)
     A DNA sequence coding for an N-terminal methionine
and amino acids 27 through 48 of human OPG followed by
amino acid residues 49 through 401 of murine OPG was
placed under control of the lux PR promoter of
prokaryotic expression vector pAMG21 as follows.
Purified plasmid DNA of pAMG21-huOPG-met[27-401] (See
Section J) was cleaved with AatII and KpnI restriction
endonucleases and a ~1075 bp B fragment isolated from
an agarose gel using standard recombinant DNA
methodology. Additionally, plasmid pAMG21-muOPG-met[22-
401] DNA (See Section D) was digested with KpnI and
BamHI restriction endonucleases and the ~1064 bp B
fragment isolated as described above. The isolated
~1075 bp pAMG21-huOPG-met[27-401] restriction fragment
containing AatII & KpnI cohesive ends (see above), the
~1064 bp pAMG21-muOPG-met[22-401] restriction fragment
containing KpnI and BamHI sticky ends and a ~5043 bp
restriction fragment containing AatII and BamHI
cohesive ends and corresponding to the nucleic acid
sequence of pAMG21 between AatII & BamHI were ligated

using standard recombinant DNA methodology. The
ligation was transformed into E. coli host 393 by
electroporation utilizing the manufacturer's protocol.
Clones were selected, and the presence of the
5   recombinant insert in the plasmid verified using
standard DNA methodology. muOPG-27-401 (P33E, G36S,
A45P) gene. Amino acid changes in muOPG from proline-33
to glutamic acid-33, glycine-36 to serine-36, and
alanine-45 to proline-45, result from replacement of
10 muOPG residues 27 through 48 with huOPG residues 27
through 48.

        Expression of recombinant muOPG-met[27-401] (P33E,
G36S, A45P) from transformed 393 cells harboring the
recombinant pAMG21 plasmid was determined using methods
15 described in Section C.

R. Murine OPG met-lys-(his)7-ala-ser-(asp)4-lys[22-401]
(A45T)

        A DNA sequence coding for an N-terminal His tag
and enterokinase recognition sequence which is (NH2 to
20 COOH terminus): Met-Lys-His-His-His-His-His-His-His-
Ala-Ser-Asp-Asp-Asp-Asp-Lys (=HEK), followed by amino
acids 22 through 401 of the murine OPG polypeptide was
placed under control of the lac repressor regulated Ps4
promoter as follows. pAMG22-His (See Section A) was
25 digested with NheI and BamHI restriction endonucleases,
and the large fragment (the A fragment) isolated from
an agarose gel using standard recombinant DNA
methodology. Oligonucleotides #1282-91 and #1282-92
were phosphorylated individually and allowed to form an
30 oligo linker duplex using methods previously described
(See Section B). The phosphorylated linker duplex
formed between oligos #1282-91 and #1282-92 containing
NheI and KpnI cohesive ends, the KpnI and BamHI
digested and purified PCR product described (see
35 Section D), and the A fragment of vector pAMG22-His

digested with NheI and BamHI were ligated using
standard recombinant DNA methodology. The ligation was
transformed into E. coli host GM120 by electroporation
utilizing the manufacturer's protocol. Clones were
5   selected, plasmid DNA isolated and DNA sequencing
performed to verify the DNA sequence of the muOPG-
HEK[22-401] gene. DNA sequencing revealed a spurious
mutation in the natural muOPG sequence that resulted in
a single amino acid change of Alanine-45 of muOPG
10 polypeptide to a Threonine.

     Expression of recombinant muOPG-HEK[22-401] (A45T)
from GM120 cells harboring the recombinant pAMG21
plasmid was determined using methods similar to those
described in Section C, except instead of addition of
15 the synthetic autoinducer, IPTG was added to 0.4 mM
final to achieve induction.

     Oligo #1282-91:
5'-CTA GCG ACG ACG ACG ACA AAG AAA CTC TGC CTC CAA AAT ACC TGC ATT
ACG ATC CGG AAA CTG GTC ATC AGC TGC TGT GTG ATA AAT GTG CTC CGG
20 GTA C-3' (SEQ ID NO:91)

     Oligo #1282-92:
5'-CCG GAG CAC ATT TAT CAC ACA GCA GCT GAT GAC CAG TTT CCG GAT CGT
AAT GCA GGT ATT TTG GAG GCA GAG TTT CTT TGT CGT CGT CGT CG-3'
(SEQ ID NO:92)

25 S. Human OPG met-arg-gly-ser-(his)6[22-401]

     Eight oligonucleotides (1338-09 to 1338-16 shown
below) were designed to produce a 175 base fragment as
overlapping, double stranded DNA. The oligos were
annealed, ligated, and the 5' and 3' oligos were used
30 as PCR primers to produce large quantities of the 175
base fragment. The final PCR gene products were
digested with restriction endonucleases ClaI and KpnI
to yield a fragment which replaces the N-terminal 28
codons of human OPG. The ClaI and KpnI digested PCR
35 product was inserted into pAMG21-huOPG [27-401] which
had also been cleaved with ClaI and KpnI. Ligated DNA

was transformed into competent host cells of E. coli
strain 393. Clones were screened for the ability to
produce the recombinant protein product and to possess
the gene fusion having the correct nucleotide sequence.
5   Protein expression levels were determined from 50 ml
shaker flask studies. Whole cell lysate and sonic
pellet were analyzed for expression of the construct by
Coomassie stained PAGE gels and Western analysis with
murine anti-OPG antibody. Expression of huOPG Met-Arg-
10   Gly-Ser-(His)$_6$ [22-401] resulting in the formation of
large inclusion bodies and the protein was localized to
the insoluble (pellet) fraction.

        1338-09:
     ACA AAC ACA ATC GAT TTG ATA CTA GA (SEQ ID NO:93)
15   1338-10:
     TTT GTT TTA ACT AAT TAA AGG AGG AAT AAA ATA TGA GAG GAT CGC ATC AC
     (SEQ ID NO:94)
         1338-11:
     CAT CAC CAT CAC GAA ACC TTC CCG CCG AAA TAC CTG CAC TAC GAC GAA GA
20   (SEQ ID NO:95)
         1338-12:
     AAC CTC CCA CCA GCT GCT GTG CGA CAA ATG CCC GCC GGG TAC CCA AAC A
     (SEQ ID NO:96)
         1338-13:
25   TGT TTG GGT ACC CGG CGG GCA TTT GT (SEQ ID NO:97)
         1338-14:
     CGC ACA GCA GCT GGT GGG AGG TTT CTT CGT CGT AGT GCA GGT ATT TCG GC
     (SEQ ID NO:98)
         1338-15:
30   GGG AAG GTT TCG TGA TGG TGA TGG TGA TGC GAT CCT CTC ATA TTT TAT T
     (SEQ ID NO:99)
         1338-16:
     CCT CCT TTA ATT AGT TAA AAC AAA TCT AGT ATC AAA TCG ATT GTG TTT GT
     (SEQ ID NO:100)
35   T. Human OPG met-lys[22-401] and met(lys)3[22-401]

        To construct the met-lys and met-(lys)3 versions
of human OPG[22-401], overlapping oligonucleotides were
designed to add the appropriate number of lysine

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residues. The two oligos for each construct were
designed to overlap, allowing two rounds of PCR to
produce the final product. The template for the first
PCR reaction was a plasmid DNA preparation containing
5   the human OPG 22-401 gene. The first PCR added the
lysine residue(s). The second PCR used the product of
the first round and added sequence back to the first
restriction site, ClaI.
        The final PCR gene products were digested with
10   restriction endonucleases ClaI and KpnI, which replace
the N-terminal 28 codons of hu OPG, and then ligated
into plasmid pAMG21-hu OPG [27-401] which had been also
digested with the two restriction endonucleases.
Ligated DNA was transformed into competent host cells
15   of E. coli strain 393. Clones were screened for the
ability to produce the recombinant protein product and
to possess the gene fusion having the correct
nucleotide sequence. Protein expression levels were
determined from 50 ml shaker flask studies. Whole cell
20   lysate and sonic pellet were analyzed for expression of
the construct by Coomassie stained PAGE gels and
Western analysis with murine anti-OPG antibody. Neither
construct had a detectable level of protein expression
and inclusion bodies were not visible. The DNA
25   sequences were confirmed by DNA sequencing.
Oligonucleotide primers to prepare Met-Lys huOPG[22-
401]:
        1338-17:
ACA AAC ACA ATC GAT TTG ATA CTA GAT TTG TTT TAA CTA ATT AAA GGA
30   GGA ATA AAA TG (SEQ ID NO:101)
        1338-18:
CTA ATT AAA GGA GGA ATA AAA TGA AAG AAA CTT TTC CTC CAA AAT ATC
(SEQ ID NO:102)
        1338-20:
35   TGT TTG GGT ACC CGG CGG ACA TTT ATC ACA C (SEQ ID NO:103)

Oligonucleotide primers to prepare Met-(Lys)$_3$-huOPG[22-401]:

1338-17:

ACA AAC ACA ATC GAT TTG ATA CTA GAT TTG TTT TAA CTA ATT AAA GGA

GGA ATA AAA TG (SEQ ID NO:104)

1338-19:

CTA ATT AAA GGA GGA ATA AAA TGA AAA AAA AAG AAA CTT TTC CTC CAA

AAT ATC (SEQ ID NO:105)

1338-20:

TGT TTG GGT ACC CGG CGG ACA TTT ATC ACA C (SEQ ID NO:106)

U. Human and Murine OPG [22-401]/Fc Fusions

Four OPG-Fc fusions were constructed where the Fc region of human IgG1 was fused at the N-terminus of either human or murine Osteoprotegerin amino acids 22 to 401 (referred to as Fc/OPG [22-401]) or at the C-terminus (referred to as OPG[22-401]/Fc). Fc fusions were constructed using the fusion vector pFc-A3 described in Example 7.

All fusion genes were constructed using standard PCR technology. Template for PCR reactions were plasmid preparations containing the target genes. Overlapping oligos were designed to combine the C-terminal portion of one gene with the N terminal portion of the other gene. This process allows fusing the two genes together in the correct reading frame after the appropriate PCR reactions have been performed. Initially one "fusion" oligo for each gene was put into a PCR reaction with a universal primer for the vector carrying the target gene. The complimentary "fusion" oligo was used with a universal primer to PCR the other gene. At the end of this first PCR reaction, two separate products were obtained, with each individual gene having the fusion site present, creating enough overlap to drive the second round of PCR and create the desired fusion. In the second round of PCR, the first two PCR products were combined along with universal primers and via the

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overlapping regions, the full length fusion DNA
sequence was produced.

The final PCR gene products were digested with
restriction endonucleases XbaI and BamHI, and then
5   ligated into the vector pAMG21 having been also
digested with the two restriction endonucleases.
Ligated DNA was transformed into competent host cells
of E. coli strain 393. Clones were screened for the
ability to produce the recombinant protein product and
10 to possess the gene fusion having the correct
nucleotide sequence. Protein expression levels were
determined from 50 ml shaker flask studies. Whole cell
lysate, sonic pellet, and supernatant were analyzed for
expression of the fusion by Coomassie stained PAGE gels
15 and Western analysis with murine anti-OPG antibody.

Fc/huOPG [22-401]

Expression of the Fc/hu OPG [22-401] fusion
peptide was detected on a Coomassie stained PAGE gel
and on a Western blot. The cells have very large
20 inclusion bodies, and the majority of the product is in
the insoluble (pellet) fraction. The following primers
were used to construct this OPG-Fc fusion:

1318-48:

CAG CCC GGG TAA AAT GGA AAC GTT TCC TCC AAA ATA TCT TCA TT
25 (SEQ ID NO:107)

1318-49:

CGT TTC CAT TTT ACC CGG GCT GAG CGA GAG GCT CTT CTG CGT GT
(SEQ ID NO:108)

Fc/muOPG [22-401]

30 Expression of the fusion peptide was detected on a
Coomassie stained gel and on a Western blot. The cells
have very large inclusion bodies, and the majority of
the product is in the insoluble (pellet) fraction. The
following primers were used to construct this OPG-Fc
35 fusion:

1318-50:

CGC TCA GCC CGG GTA AAA TGG AAA CGT TGC CTC CAA AAT ACC TGC

(SEQ ID NO:109)

    1318-51:

CCA TTT TAC CCG GGC TGA GCG AGA GGC TCT TCT GCG TGT

5   (SEQ ID NO:110)

muOPG [22-401]/Fc

    Expression of the fusion peptide was detected on a
Coomassie stained gel and on a Western blot. The amount
of recombinant product was less than the OPG fusion

10   proteins having the Fc region in the N terminal
position. Obvious inclusion bodies were not detected.
Most of the product appeared to be in the insoluble
(pellet) fraction. The following primers were used to
construct this OPG-Fc fusion:

15   1318-54:

GAA AAT AAG CTG CTT AGC TGC AGC TGA ACC AAA ATC (SEQ ID NO:111)

    1318-55:

CAG CTG CAG CTA AGC AGC TTA TTT TCA CGG ATT G (SEQ ID NO:112)

huOPG [22-401]/Fc

20   Expression of the fusion peptide was not detected
on a Coomassie stained gel, although a faint Western
positive signal was present. Obvious inclusion bodies
were not detected. The following primers were used to
prepare this OPG-Fc fusion:

25   1318-52:

AAA AAT AAG CTG CTT AGC TGC AGC TGA ACC AAA ATC (SEQ ID NO:113)

    1318-53:

CAG CTG CAG CTA AGC AGC TTA TTT TTA CTG ATT GG (SEQ ID NO:114)

V. Human OPG met[22-401]-Fc fusion (P25A)

30   This construct combines a proline to alanine amino
acid change at position 25 (P25A) with the huOPG
met[22-401]-Fc fusion. The plasmid was digested with
restriction endonucleases ClaI and KpnI, which removes
the N-terminal 28 codons of the gene, and the resulting

35   small (less than 200 base pair) fragment was gel
purified. This fragment containing the proline to

alanine change was then ligated into plasmid pAMG21-
huOPG [22-401]-Fc fusion which had been digested with
the two restriction endonucleases. The ligated DNA was
transformed into competent host cells of E. coli strain
5   393. Clones were screened for the ability to produce
the recombinant protein product and to possess the gene
fusion having the correct nucleotide sequence. Protein
expression levels were determined from 50 ml shaker
flask studies. Whole cell lysate and sonic pellet were
10 analyzed for expression of the construct by Coomassie
stained PAGE gels and Western analysis with murine
anti-OPG antibody. The expression level of the fusion
peptide was detected on a Coomassie stained PAGE gel
and on a Western blot. The protein was in the insoluble
15 (pellet) fraction. The cells had large inclusion
bodies.

W. Human OPG met[22-401] (P25A)

     A DNA sequence coding for an N-terminal methionine
and amino acids 22 through 401 of human OPG with the
20 proline at position 25 being substituted by alanine
under control of the lux P$_R$ promoter in prokaryotic
expression vector pAMG21 was constructed as follows:
Synthetic oligos # 1289-84 and 1289-85 were annealed to
form an oligo linker duplex with XbaI and KpnI cohesive
25 ends. The synthetic linker duplex utilized optimal E.
coli codons and encoded an N-terminal methionine. The
linker also included an SpeI restriction site which was
not present in the original sequence. The linker duplex
was directionally inserted between the XbaI and KpnI
30 sites in pAMG21-huOPG-22-401 using standard methods.
The ligation mixture was introduced into E. coli host
GM221 by transformation. Clones were initially screened
for production of the recombinant protein. Plasmid DNA
was isolated from positive clones and DNA sequencing
35 was performed to verify the DNA sequence of the HuOPG-

Met[22-401](P25A) gene. The following oligonucleotides
were used to generate the XbaI - KpnI linker:

Oligo #1289-84:

5'-CTA GAA GGA GGA ATA ACA TAT GGA AAC TTT TGC TCC AAA ATA TCT TCA

5 TTA TGA TGA AGA AAC TAG TCA TCA GCT GCT GTG TGA TAA ATG TCC GCC

GGG TAC -3' (SEQ ID NO:115)

Oligo #1289-85:

5'-CCG GCG GAC ATT TAT CAC ACA GCA GCT GAT GAC TAG TTT CTT CAT CAT

AAT GAA GAT ATT TTG GAG CAA AAG TTT CCA TAT GTT ATT CCT CCT T-3'

10 (SEQ ID NO:116)

X. Human OPG met[22-401] (P26A) and (P26D)

A DNA sequence coding for an N-terminal methionine
and amino acids 22 through 401 of human OPG with the
proline at position 26 being substituted by alanine

15 under control of the lux P_R promoter in prokaryotic
expression vector pAMG21 was constructed as follows:
Synthetic oligos # 1289-86 and 1289-87 were annealed to
form an oligo linker duplex with XbaI and SpeI cohesive
ends. The synthetic linker duplex utilized optimal E.

20 coli codons and encoded an N-terminal methionine. The
linker duplex was directionally inserted between the
XbaI and SpeI sites in pAMG21-huOPG[22-401](P25A) using
standard methods. The ligation mixture was introduced
into E. coli host GM221 by transformation. Clones were

25 initially screened for production of the recombinant
protein. Plasmid DNA was isolated from positive clones
and DNA sequencing was performed to verify the DNA
sequence of the huOPG-met[22-401](P26A) gene. One of
the clones sequenced was found to have the proline at

30 position 26 substituted by aspartic acid rather than
alanine, and this clone was designated huOPG-met[22-
401](P26D). The following oligonucleotides were used to
generate the XbaI - SpeI linker:

Oligo #1289-86:

35 5' - CTA GAA GGA GGA ATA ACA TAT GGA AAC TTT TCC TGC TAA ATA TCT

TCA TTA TGA TGA AGA AA - 3' (SEQ ID NO:117)

Oligo #1289-87:

5' - CTA GTT TCT TCA TCA TAA TGA AGA TAT TTA GCA GGA AAA GTT TCC
ATA TGT TAT TCC TCC TT - 3' (SEQ ID NO:118)

Y. Human OPG met[22-194] (P25A)

5   A DNA sequence coding for an N-terminal methionine
and amino acids 22 through 194 of human OPG with the
proline at position 25 being substituted by alanine
under control of the lux P_R promoter in prokaryotic
expression vector pAMG21 was constructed as follows:

10   The plasmids pAMG21-huOPG[27-194] and pAMG21-huOPG[22-
401] (P25A) were each digested with KpnI and BamHI
endonucleases. The 450 bp fragment was isolated from
pAMG21-huOPG[27-194] and the 6.1 kbp fragment was
isolated from pAMG21-huOPG[22-401] (P25A). These

15   fragments were ligated together and introduced into E.
coli host GM221 by transformation. Clones were
initially screened for production of the recombinant
protein. Plasmid DNA was isolated from positive clones
and DNA sequencing was performed to verify the DNA

20   sequence of the huOPG-Met[22-194](P25A) gene.

EXAMPLE 9

Association of OPG Monomers

CHO cells engineered to overexpress muOPG [22-401]
were used to generate conditioned media for the

25   analysis of secreted recombinant OPG using rabbit
polyclonal anti-OPG antibodies. An aliquot of
conditioned media was concentrated 20-fold, then
analysed by reducing and non-reducing SDS-PAGE
(Figure 15). Under reducing conditions, the protein

30   migrated as a Mr 50-55 kd polypeptide, as would be
predicted if the mature product was glycosylated at one
or more of its consensus N-linked glycosylation sites.
Suprisingly, when the same samples were analysed by
non-reducing SDS-PAGE, the majority of the protein

35   migrated as an approximately 100 kd polypeptide, twice
the size of the reduced protein. In addition, there was

a smaller amount of the Mr 50-55 kd polypeptide. This
pattern of migration on SDS-PAGE was consistent with
the notion that the OPG product was forming dimers
through oxidation of a free sulfhydryl group(s).

5   The predicted mature OPG polypeptide contains 23
cysteine residues, 18 of which are predicted to be
involved in forming intrachain disulfide bridges which
comprise the four cysteine-rich domains (Figure 12A).
The five remaining C-terminal cysteine residues are not

10   involved in secondary structure which can be predicted
based upon homology with other TNFR family members.
Overall there is a net uneven number of cysteine
residues, and it is formally possible that at least one
residue is free to form an intermolecular disulfide

15   bond between two OPG monomers.

        To help elucidate patterns of OPG kinesis and
monomer association, a pulse-chase labelling study was
performed. CHO cells expressing muOPG [22-401] were
metabolically labelled as described above in serum-free

20   medium containing ^{35}S methionine and cysteine for 30
min. After this period, the media was removed, and
replaced with complete medium containing unlabelled
methionine and cysteine at levels approximately 2,000-
fold excess to the original concentration of

25   radioactive amino acids. At 30 min, 1hr, 2 hr, 4 hr, 6
hr and 12 hr post addition, cultures were harvested by
the removal of the conditioned media, and lysates of
the conditioned media and adherent monolayers were
prepared. The culture media and cell lysates were

30   clarified as described above, and then
immunoprecipitated using anti-OPG antibodies as
described above. After the immunoprecipitates were
washed, they were released by boiling in non-reducing
SDS-PAGE buffer then split into two equal halves. To

35   one half, the reducing agent β-mercaptothanol was added

to 5% (v/v) final concentration, while the other half
was maintained in non-reducing conditions. Both sets of
immunoprecipitates were analysed by SDS-PAGE as
described above, then processed for autoradiography and
5   exposed to film. The results are shown in Figure 16.
The samples analysed by reducing SDS-PAGE are depicted
in the bottom two panels. After synthesis, the OPG
polypeptide is rapidly processed to a slightly larger
polypeptide, which probably represents modification by
10 N-linked glycoslyation. After approximately 1-2 hours,
the level of OPG in the cell decreases dramatically,
and concomitantly appears in the culture supernatant.
This appears to be the result of the vectoral transport
of OPG from the cell into the media over time,
15 consistent with the notion that OPG is a naturally
secreted protein. Analysis of the same
immunoprecipitates under nonreducing conditions reveals
the relationship between the formation of OPG dimers
and secretion into the conditioned media (Figure 16,
20 upper panels). In the first 30-60 minutes, OPG monomers
are processed in the cell by apparent glycoslylation,
followed by dimer formation. Over time, the bulk of OPG
monomers are driven into dimers, which subsequently
disappear from the cell. Beginning about 60 minutes
25 after synthesis, OPG dimers appear in the conditioned
media, and accumulate over the duration of the
experiment. Following this period, OPG dimers are
formed, which are then secreted into the culture media.
OPG monomers persist at a low level inside the cell
30 over time, and small amounts also appear in the media.
This does not appear to be the result of breakdown of
covalent OPG dimers, but rather the production of sub-
stoichiometric amounts of monomers in the cell and
subsequent secretion.
35   Recombinantly produced OPG from transfected CHO
cells appears to be predominantly a dimer. To determine

if dimerization is a natural process in OPG synthesis, we analysed the conditioned media of a cell line found to naturally express OPG. The CTLL-2 cell line, a murine cytotoxic T lymphocytic cell line (ATCC accession no. TIB-214), was found to express OPG mRNA in a screen of tissue and cell line RNA. The OPG transcript was found to be the same as the cloned and sequenced 2.5-3.0 kb RNA identified from kidney and found to encode a secreted molecule. Western blot analysis of conditioned media obtained from CTLL-2 cells shows that most, if not all, of the OPG secreted is a dimer (Figure 17). This suggests that OPG dimerization and secretion is not an artifact of overexpression in a cell line, but is likely to be the main form of the product as it is produced by expressing cells.

Normal and transgenic mouse tissues and serum were analysed to determine the nature of the OPG molecule expressed in OPG transgenic mice. Since the rat OPG cDNA was expressed under the control of a hepatocyte control element, extracts made from the parenchyma of control and transgenic mice under non-reducing conditions were analysed (Figure 18). In extract from transgenic, but not control mice, OPG dimers are readily detected, along with substoichiometric amounts of monomers. The OPG dimers and monomers appear identical to the recombinant murine protein expressed in the genetically engineered CHO cells. This strongly suggests that OPG dimers are indeed a natural form of the gene product, and are likely to be key active components. Serum samples obtained from control and transgenic mice were similarly analysed by western blot analysis. In control mice, the majority of OPG migrates as a dimer, while small amounts of monomer are also detected. In addition, significant amounts of a larger OPG related protein is detected, which migrates with a

relative molecular mass consistent with the predicted
size of a covalently-linked trimer. Thus, recombinant
OPG is expressed predominantly as a dimeric protein in
OPG transgenic mice, and the dimer form may be the
5   basis for the osteopetrotic phenotype in OPG mice. OPG
recombinant protein may also exist in higher molecular
weight "trimeric" forms.

To determine if the five C-terminal cysteine
residues of OPG play a role in homodimerization, the
10   murine OPG codons for cytsteine residues 195 (C195),
C202, C277, C319, and C400 were changed to serine using
the QuickChange™ Site-Directed Mutagenesis Kit
(Stratagene, San Diego, CA) as described above. The
muOPG gene was subcloned between the Not I and Xba I
15   sites of the pcDNA 3.1 (+) vector (Invitrogen, San
Diego, CA). The resulting plasmid, pcDNA3.1-muOPG, and
mutagenic primers were treated with Pfu polymerase in
the presence of deoxynucleotides, then amplified in a
thermocycler as described above. An aliqout of the
20   reaction is then transfected into competent E. coli
XL1-Blue by heatshock, then plated. Plasmid DNA from
transformants was then sequenced to verify mutations.

The following primer pairs were used to change the
codon for cysteine residue 195 to serine of the murine
25   OPG gene, resulting in the production of a muOPG [22-
401] C195S protein:

 1389-19:
5'-CAC GCA AAA GTC GGG AAT AGA TGT CAC-3' (SEQ ID NO:150)
1406-38:
30   5'-GTG ACA TCT ATT CCC GAC TTT TGC GTG-3' (SEQ ID NO:151)

The following primer pairs were used to change the
codon for cysteine residue 202 to serine of the murine
OPG gene, resulting in the production of a muOPG [22-
401] C202S protein:
35   1389-21:
5'-CAC CCT GTC GGA AGA GGC CTT CTT C-3' (SEQ ID NO:152)

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1389-22:

5'-GAA GAA GGC CTC TTC CGA CAG GGT G-3' (SEQ ID NO:153)

The following primer pairs were used to change the codon for cysteine residue 277 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C277S protein:

1389-23:

5'-TGA CCT CTC GGA AAG CAG CGT GCA-3' (SEQ ID NO:154)

1389-24:

5'-TGC ACG CTG CTT TCC GAG AGG TCA-3' (SEQ ID NO:155)

The following primer pairs were used to change the codon for cysteine residue 319 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C319S protein:

1389-17:

5'-CCT CGA AAT CGA GCG AGC AGC TCC-3' (SEQ ID NO:156)

1389-18:

5'-CGA TTT CGA GGT CTT TCT CGT CT C-3' (SEQ ID NO:157)

The following primer pairs were used to change the codon for cysteine residue 400 to serine of the murine OPG gene, resulting in the production of a muOPG [22-401] C400S protein:

1406-72:

5'-CCG TGA AAA TAA GCT CGT TAT AAC TAG GAA TGG-3' (SEQ ID NO:158)

1406-75:

5'-CCA TTC CTA GTT ATA ACG AGC TTA TTT TCA CGG-3' (SEQ ID NO:159)

Each resulting muOPG [22-401] plasmid containing the appropriate mutation was then transfected into human 293 cells, the mutant OPG-Fc fusion protein purified from conditioned media as described above. The biological activity of each protein was assessed the in vitro osteoclast forming assay described in example 11. Conditioned media from each transfectant was analysed

by non-reducing SDS-PAGE and western blotting with
anti-OPG antibodies.

    Mutation of any of the five C-terminal cysteine
residues results in the production of predominantly
(>90%) monomeric 55 kd OPG molecules. This strongly
suggests that the C-terminal cysteine residues together
play a role in OPG homodimerization.

    C-terminal OPG deletion mutants were constructed
to map the region(s) of the OPG C-terminal domain which
are important for OPG homodimerization. These OPG
mutants were constructed by PCR amplification using
primers which introduce premature stop translation
signals in the C-terminal region of murine OPG. The 5′
oligo was designed to the MuOPG start codon (containing
a HindIII restriction site) and the 3′ oligonucleotides
(containing a stop codon and XhoI site) were designed
to truncate the C-terminal region of muOPG ending at
either threonine residue 200 (CT 200), proline 212
(CT212), glutamic acid 293 (CT-293), or serine 355 (CT-
355).

    The following primers were used to construct muOPG
[22-200]:

    1091-39:

5′ -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3′
(SEQ ID NO:160)

    1391-91:

5′ -CCT CTC TCG AGT CAG GTG ACA TCT ATT CCA CAC TTT TGC GTG GC-3′
(SEQ ID NO:161)

    The following primers were used to construct muOPG
[22-212]:

    1091-39:

5′ -CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3′
(SEQ ID NO:162)

    1391-90:

5′ -CCT CTC TCG AGT CAA GGA ACA GCA AAC CTG AAG AAG GC -3′
(SEQ ID NO:163)

The following primers were used to construct muOPG [22-293]:

1091-39:

5'-CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3' (SEQ ID NO:164)

1391-89:

5'- CCT CTC TCG AGT CAC TCT GTG GTG AGG TTC GAG TGG CC-3' (SEQ ID NO:165)

The following primers were used to construct muOPG [22-355]:

1091-39:

5'-CCT CTG AGC TCA AGC TTC CGA GGA CCA CAA TGA ACA AG-3' (SEQ ID NO:166)

1391-88:

5' CCT CTC TCG AGT CAG GAT GTT TTC AAG TGC TTG AGG GC-3' (SEQ ID NO:167)

Each resulting muOPG-CT plasmid containing the appropriate truncation was then transfected into human 293 cells, the mutant OPG-Fc fusion protein purified from conditioned media as described above. The biological activity of each protein was assessed the in vitro osteoclast forming assay described in example 11. The conditioned medias were also analysed by non-reducing SDS-PAGE and western blotting using anti-OPG antibodies.

Truncation of the C-terminal region of OPG effects the ability of OPG to form homodimers. CT 355 is predominantly monomeric, although some dimer is formed. CT 293 forms what appears to be equal molar amounts of monomer and dimer, and also high molecular weight aggregates. However, CT 212 and CT 200 are monomeric.

EXAMPLE 10

Purification of OPG

A. Purification of mammalian OPG-Fc Fusion Proteins

5 L of conditioned media from 293 cells expressing an OPG-Fc fusion protein were prepared as follows. A

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frozen sample of cells was thawed into 10 ml of 293S
media (DMEM-high glucose, 1x L-glutamine, 10% heat
inactivated fetal bovine serum (FBS) and 100 ug/ml
hygromycin) and fed with fresh media after one day.
5 After three days, cells were split into two T175 flasks
at 1:10 and 1:20 dilutions. Two additional 1:10 splits
were done to scale up to 200 T175 flasks. Cells were at
5 days post-thawing at this point. Cells were grown to
near confluency (about three days) at which time serum-
10 containing media was aspirated, cells were washed one
time with 25 ml PBS per flask and 25 ml of SF media
(DMEM-high glucose, 1x L-glutamine) was added to each
flask. Cells were maintained at 5% CO_2 for three days
at which point the media was harvested, centrifuged,
15 and filtered through 0.45m cellulose nitrate filters
(Corning).

     OPG-Fc fusion proteins were purified using a
Protein G Sepharose column (Pharmacia) equilibrated in
PBS. The column size varied depending on volume of
20 starting media. Conditioned media prepared as described
above was loaded onto the column, the column washed
with PBS, and pure protein eluted using 100mM glycine
pH 2.7. Fractions were collected into tubes containing
1M Tris pH 9.2 in order to neutralize as quickly as
25 possible. Protein containing fractions were pooled,
concentrated in either an Amicon Centricon 10 or
Centriprep 10 and diafiltered into PBS. The pure
protein is stored at -80°C.

     Murine [22-401]-Fc, Murine [22-180]-Fc, Murine
30 [22-194]-Fc, human [22-401]-Fc and human [22-201]Fc
were purified by this procedure. Murine [22-185]-Fc is
purified by this procedure.

B. Preparation of anti-OPG antibodies

     Three New Zealand White rabbits (5-8 lbs initial
35 wt) were injected subcutaneously with muOPG[22-401]-Fc
fusion protein. Each rabbit was immunized on day 1 with

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50 µg of antigen emulsified in an equal volume of
Freunds complete adjuvant. Further boosts (Days 14 and
28) were performed by the same procedure with the
substitution of Freunds incomplete adjuvant. Antibody
5   titers were monitored by EIA. After the second boost,
the antisera revealed high antibody titers and 25ml
production bleeds were obtained from each animal. The
sera was first passed over an affinity column to which
murine OPG-Fc had be immobilized. The anti-OPG
10 antibodies were eluted with Pierce Gentle Elution
Buffer containing 1% glacial acetic acid. The eluted
protein was then dialyzed into PBS and passed over a Fc
column to remove any antibodies specific for the Fc
portion of the OPG fusion protein. The run through
15 fractions containing anti-OPG specific antibodies were
dialyzed into PBS.
     C. Purification of murine OPG[22-401]
          Antibody Affinity Chromatography
          Affinity purified anti-OPG antibodies were
20 diafiltered into coupling buffer (0.1M sodium carbonate
pH 8.3, 0.5M NaCl), and mixed with CNBr-activated
sepharose beads (Pharmacia) for two hours at room
temperature. The resin was then washed with coupling
buffer extensively before blocking unoccupied sited
25 with 1M ethanolamine (pH 8.0) for two hours at room
temperature. The resin was then washed with low pH
(0.1M sodium acetate pH 4.0, 0.5M NaCl) followed by a
high pH wash (0.1M Tris-HCl pH 8.0, 0.5M NaCl). The
last washes were repeated three times. The resin was
30 finally equilibrated with PBS before packing into a
column. Once packed, the resin was washed with PBS. A
blank elution was performed with 0.1M glycine-HCl,
pH 2.5), followed by re-equilibration with PBS.
          Concentrated conditioned media from CHO cells
35 expressing muOPG[22-410] was applied to the column at a

low flow rate. The column was washed with PBS until UV
absorbance measured at 280nm returned to baseline. The
protein was eluted from the column first with 0.1M
glycine-HCl (pH 2.5), re-equilibrated with PBS, and
5   eluted with a second buffer (0.1M CAPS, pH 10.5), 1M
NaCl). The two elution pools were diafiltered
separately into PBS and sterile filtered before
freezing at -20°C.

Conventional Chromatography

10   CHO cell conditioned media was concentrated 23x in
an Amicon spiral wound cartridge (S10Y10) and
diafiltered into 20mM tris pH 8.0. The diafiltered
media was then applied to a Q-sepharose HP (Pharmacia)
column which had been equilibrated with 20mM tris pH
15   8.0. The column was then washed until absorbence at 280
nm reached baseline. Protein was eluted with a 20
column volume gradient of 0-300 mM NaCl in tris pH 8.0.
OPG was detected using a western blot of column
fractions.

20   Fractions containing OPG were pooled and brought
to a final concentration of 300 mM NaCl, 0.2 mM DTT. A
NiNTA superose (Qiagen) column was equilibrated with
20mM tris pH 8.0, 300 mM NaCl, 0.2 mM DTT after which
the pooled fractions were applied. The column was
25   washed with equilibration buffer until baseline
absorbence was reached. Proteins were eluted from the
column with a 0-30mM Imidazole gradient in
equilibration buffer. Remaining proteins were washed
off the column with 1M Imidazole. Again a western blot
30   was used to detect OPG containing fractions.

Pooled fractions from the NiNTA column were
dialyzed into 10 mm potassium phosphate pH 7.0, 0.2mM
DTT. The dialyzed pool was then applied to a ceramic
hydroxyapatite column (Bio-Rad) which had been
35   equilibrated in 10mM phosphate buffer. After column
washing, the protein was eluted with a 10-100 mM

potassium phosphate gradient over 20 column volumes.
This was then followed by a 20 column volume gradient
of 100-400 mM phosphate.

5   OPG was detected by coomassie blue staining of
SDS-polyacrylamide gels and by western blotting.
Fractions were pooled and diafiltered onto PBS and
frozen at -80°C. The purified protein runs as a monomer
and will remain so after diafiltration into PBS. The
monomer is stable when stored frozen or at pH 5 at 4°C.

10   However if stored at 4°C in PBS, dimers and what
appears to be trimers and tetramers will form after one
week.

    D. <u>Purification of human OPG met[22-401] from E. coli</u>
     The bacterial cell paste was suspended into 10 mM

15   EDTA to a concentration of 15% (w/v) using a low shear
homogenizer at 5°C. The cells were then disrupted by
two homogenizations at 15,000 psi each at 5°C. The
resulting homogenate was centrifuged at 5,000 x g for
one hour at 5°C. The centrifugal pellet was washed by

20   low shear homogenization into water at the original
homogenization volume followed by centrifugation as
before. The washed pellet was then solubilized to 15%
(w/v) by a solution of (final concentration) 6 M
guanidine HCl, 10 mM dithiothreitol, 10 mM TrisHCl, pH

25   8.5 at ambient temperature for 30 minutes. This
solution was diluted 30-fold into 2M urea containing 50
mM CAPS, pH 10.5, 1 mM reduced glutathione and then
stirred for 72 hours at 5°C. The OPG was purified from
this solution at 25°C by first adjustment to pH 4.5

30   with acetic acid and then chromatography over a column
of SP-HP Sepharose resin equilibrated with 25 mM sodium
acetate, pH 4.5. The column elution was carried out
with a linear sodium chloride gradient from 50 mM to
550 mM in the same buffer using 20 column volumes at a

35   flow rate of 0.1 column volumes/minute. The peak
fractions containing only the desired OPG form were

pooled and stored at 5°C or buffer exchanged into
phosphate buffered saline, concentrated by
ultrafiltration, and then stored at 5°C. This material
was analyzed by reverse phase HPLC, SDS-PAGE, limulus
5   amebocyte lysate assay for the presence of endotoxin,
and N-terminal sequencing. In addition, techniques such
as mass spectrometry, pH/temperature stability,
fluoresence, circular dichroism, differential scanning
calorimetry, and protease profiling assays may also be
10 used to examine the folded nature of the protein.

EXAMPLE 11

Biological Activity of Recombinant OPG

Based on histology and histomorphometry, it
appeared that hepatic overexpression of OPG in
15 transgenic mice markedly decreased the numbers of
osteoclasts leading to a marked increase in bone tissue
(see Example 4). To gain further insight into potential
mechanism(s) underlying this in vivo effect, various
forms of recombinant OPG have been tested in an in
20 vitro culture model of osteoclast formation (osteoclast
forming assay). This culture system was originally
devised by Udagawa (Udagawa et al. Endocrinology 125,
1805-1813 (1989), Proc. Natl. Acad. Sci. USA 87, 7260-
7264 (1990)) and employs a combination of bone marrow
25 cells and cells from bone marrow stromal cell lines. A
description of the modification of this culture system
used for these studies has been previously published
(Lacey et al. Endocrinology 136, 2367-2376 (1995)). In
this method, bone marrow cells, flushed from the femurs
30 and tibiae of mice, are cultured overnight in culture
media (alpha MEM with 10% heat inactivated fetal bovine
serum) supplemented with 500 U/ml CSF-1 (colony
stimulating factor 1, also called M-CSF), a
hematopoietic growth factor specific for cells of the
35 monocyte/macrophage family lineage. Following this
incubation, the non-adherent cells are collected,

subjected to gradient purification, and then cocultured
with cells from the bone marrow cell line ST2 (1×10^6
non-adherent cells : 1×10^5 ST2 cells/ ml media). The
media is supplemented with dexamethasone (100 nM) and
5   the biologically-active metabolite of vitamin D3 known
as 1,25 dihydroxyvitamin D3 (1,25 (OH)2 D3, 10 nM). To
enhance osteoclast appearance, prostaglandin E2 (250
nM) is added to some cultures. The coculture period
usually ranges from 8 - 10 days and the media, with all
10   of the supplements freshly added, is renewed every 3-4
days. At various intervals, the cultures are assessed
for the presence of tartrate acid phosphatase (TRAP)
using either a histochemical stain (Sigma Kit # 387A,
Sigma, St. Louis, MO) or TRAP solution assay. The TRAP
15   histochemical method allows for the identification of
osteoclasts phenotypically which are multinucleated (•
3 nuclei) cells that are also TRAP+. The solution assay
involves lysing the osteoclast-containing cultures in a
citrate buffer (100 mM, pH 5.0) containing 0.1% Triton
20   X-100. Tartrate resistant acid phosphatase activity is
then measured based on the conversion of
p-nitrophenylphosphate (20 nM) to p-nitrophenol in the
presence of 80 mM sodium tartrate which occurs during a
3-5 minute incubation at RT. The reaction is terminated
25   by the addition of NaOH to a final concentration of 0.5
M. The optical density at 405 nm is measured and the
results are plotted.

    Previous studies (Udagawa et al. ibid) using the
osteoclast forming assay have demonstrated that these
30   cells express receptors for ^{125}I-calcitonin
(autoradiography) and can make pits on bone surfaces,
which when combined with TRAP positivity confirm that
the multinucleated cells have an osteoclast phenotype.
Additional evidence in support of the osteoclast
35   phenotype of the multinucleated cells that arise in

vitro in the osteoclast forming assay are that the
cells express αv and β3 integrins by
immunocytochemistry and calcitonin receptor and TRAP
mRNA by in situ hybridization (ISH).

5   The huOPG [22-401]-Fc fusion was purified from CHO
cell conditioned media and subsequently utilized in the
osteoclast forming assay. At 100 ng/ml of huOPG [22-
401]-Fc, osteoclast formation was virtually 100%
inhibited (Figure 19A). The levels of TRAP measured in

10   lysed cultures in microtitre plate wells were also
inhibited in the presence of OPG with an ID_{50} of
approximately 3 ng/ml (Figure 20). The level of TRAP
activity in lysates appeared to correlate with the
relative number of osteoclasts seen by TRAP

15   cytochemistry (compare Figures 19A-19G and 20).
Purified human IgG1 and TNF-α inhibitor were also
tested in this model and were found to have no
inhibitory or stimulatory effects suggesting that the
inhibitory effects of the huOPG [22-401]-Fc were due to

20   the OPG portion of the fusion protein. Additional forms
of the human and murine molecules have been tested and
the cumulative data are summarized in Table 3.

Table 3

Effects of various OPG forms on in vitro
osteoclast formation

OPG Construct	Relative Bioactivity in vitro
muOPG [22-401]-Fc	+++
muOPG [22-194]-Fc	+++
muOPG [22-185]-Fc	++
muOPG [22-180]-Fc	−
muOPG [22-401]	+++
muOPG [22-401] C195	+++
muOPG [22-401] C202	+
muOPG [22-401] C277	−
muOPG [22-401] C319	+
muOPG [22-401] C400	+
muOPG [22-185]	−
muOPG [22-194]	++
muOPG [22-200]	++
muOPG [22-212]	−
muOPG [22-293]	+++
muOPG [22-355]	+++
huOPG [22-401]-Fc	+++
huOPG [22-201]-Fc	+++
huOPG [22-401]-Fc P26A	+++
huOPG [22-401]-Fc Y28F	+++
huOPG [22-401]	+++
huOPG [27-401]-Fc	++
huOPG [29-401]-Fc	++
huOPG [32-401]-Fc	+/−

+++, ED_{50} = 0.4-2 ng/ml
++, ED_{50} = 2-10 ng/ml
+, ED_{50} = 10-100 ng/ml
−, ED_{50} > 100 ng/ml

The cumulative data suggest that murine and human OPG amino acid sequences 22-401 are fully active _in vitro_, when either fused to the Fc domain, or unfused. They inhibit in a dose-dependent manner and possess

5   half-maximal activities in the 2-10 ng/ml range. Truncation of the murine C-terminus at threonine residue 180 inactivates the molecule, whereas truncations at cysteine 185 and beyond have full activity. The cysteine residue located at position 185

10 is predicted to form an SS3 bond in the domain 4 region of OPG. Removal of this residue in other TNFR-related proteins has previously been shown to abrogate biological activity (Yan _et al_. (1994), _J. Biol. Chem_. 266: 12099-104). Our finding that muOPG[22-180]-Fc is

15 inactive while muOPG[22-185]-Fc is active is consistent with these findings. This suggests that amino acid residues 22-185 define a region for OPG activity.

These findings indicate that like transgenically-expressed OPG, recombinant OPG also

20 suppressed osteoclast formation as tested in the osteoclast forming assay. Time course experiments examining the appearance of TRAP+ cells, β3+ cells, F480+ cells in cultures continuously exposed to OPG demonstrate that OPG blocks the appearance TRAP+ and

25 β3+ cells, but not F480+ cells. In contrast, TRAP+ and β3+ cells begin to appear as early as day 4 following culture establishment in control cultures. Only F480+ cells can be found in OPG-treated cultures and they appear to be present at qualitatively the same numbers

30 as the control cultures. Thus, the mechanism of OPG effects in vitro appears to involve a blockade in osteoclast differentiation at a step beyond the appearance of monocyte-macrophages but before the appearance of cells expressing either TRAP or β3

35 integrins. Collectively these findings indicate that

OPG does not interfere with the general growth and
differentiation of monocyte-macrophage precursors from
bone marrow, but rather suggests that OPG specifically
blocks the selective differentiation of osteoclasts
5   from monocyte-macrophage precursors.

    To determine more specifically when in the
osteoclast differentiation pathway that OPG was
inhibitory, a variation of the in vitro culture method
was employed. This variation, described in (Lacey et
10 al. supra), employs bone marrow macrophages as
osteoclast precursors. The osteoclast precursors are
derived by taking the nonadherent bone marrow cells
after an overnight incubation in CSF-1/M-CSF, and
culturing the cells for an additional 4 days with 1,000
15 - 2,000 U/ml CSF-1. Following 4 days of culture, termed
the growth phase, the non-adherent cells are removed.
The adherent cells, which are bone marrow macrophages,
can then be exposed for up to 2 days to various
treatments in the presence of 1,000 - 2,000 U/ml CSF-1.
20 This 2 day period is called the intermediate
differentiation period. Thereafter, the cell layers are
again rinsed and then ST-2 cells (1 X 10^5 cell/ml),
dexamethasone (100 nM) and 1,25 (OH)2 D3 (10 nM) are
added for the last 8 days for what is termed the
25 terminal differentiation period. Test agents can be
added during this terminal period as well. Acquisition
of phenotypic markers of osteoclast differentiation are
acquired during this terminal period (Lacey et al.
ibid).

30   huOPG [22-401]-Fc (100 ng/ml) was tested for its
effects on osteoclast formation in this model by adding
it during either the intermediate, terminal or,
alternatively, both differentiation periods. Both TRAP
cytochemistry and solution assays were performed. The
35 results of the solution assay are shown in Figure 21.

HuOPG [22-401]-Fc inhibited the appearance of TRAP
activity when added to both the intermediate and
terminal or only the terminal differentiation phases.
When added to the intermediate phase and then removed
5   from the cultures by rinsing, huOPG [22-401]-Fc did not
block the appearance of TRAP activity in culture
lysates. The cytochemistry results parallel the
solution assay data. Collectively, these observations
indicate that huOPG [22-401]-Fc only needs to be
10 present during the terminal differentiation period for
it to exert its all of its suppressive effects on
osteoclast formation.

B. In vivo IL-1-α and IL-1-β challenge experiments

IL-1 increases bone resorption both systemically
15 and locally when injected subcutaneously over the
calvaria of mice (Boyce et al. (1989), Endocrinology
125: 1142-50). The systemic effects can be assessed by
the degree of hypercalcemia and the local effects
histologically by assessing the relative magnitude of
20 the osteoclast-mediated response. The aim of these
experiments was to determine if recombinant muOPG [22-
401]-Fc could modify the local and/or systemic actions
of IL-1 when injected subcutaneously over the same
region of the calvaria as IL-1.

25   IL-1 β experiment

Male mice (ICR Swiss white) aged 4 weeks were
divided into the following treatment groups (5 mice per
group): Control group: IL-1 treated animals (mice
received 1 injection/day of 2.5 ug of IL-1-β); Low dose
30 muOPG [22-401]-Fc treated animals (mice received 3
injections/day of 1 μg of muOPG [22-401]-Fc); Low dose
muopg [22-401]-Fc and IL-1-β; High dose muOPG [22-401]-
Fc treated animals (mice receive 3 injections/day of 10
μg muOPG [22-401]-Fc); High dose muOPG [22-401]-Fc and

IL-1-β. All mice received the same total number of
injections of either active factor or vehicle (0.1%
bovine serum albumin in phosphate buffered saline). All
groups are sacrificed on the day after the last

5   injection. The weights and blood ionized calcium levels
are measured before the first injections, four hours
after the second injection and 24 hours after the third
IL-1 injection, just before the animals were
sacrificed. After sacrifice the calvaria were removed

10   and processed for paraffin sectioning.

     IL-1 α experiment

     Male mice (ICR Swiss white) aged 4 weeks were
divided into the following treatment groups (5 mice per
group): Control group; IL-1-α treated animals (mice

15   received 1 injection/day of 5 ug of IL-1-α); Low dose
muOPG [22-401]-Fc treated animals (mice received 1
injection/day of 10 μg of muOPG [22-401]-Fc; Low dose
muopg [22-401]-Fc and IL-1-α, (dosing as above); High
dose muopg [22-401]-Fc treated animals (mice received 3

20   injections/day of 10 μg muOPG [22-401]-Fc; High dose
muOPG [22-401]-Fc and IL-1-α. All mice received the
same number of injections/day of either active factor
or vehicle. All groups were sacrificed on the day after
the last injection. The blood ionized calcium levels

25   were measured before the first injection, four hours
after the second injection and 24 hours after the third
IL-1 injection, just before the animals were
sacrificed. The animal weights were measured before the
first injection, four hours after the second injection

30   and 24 hours after the third IL-1 injection, just
before the animals were sacrificed. After sacrifice the
calvaria were removed and processed for paraffin
sectioning.

Histological methods

Calvarial bone samples were fixed in zinc
formalin, decalcified in formic acid, dehydrated
through ethanol and mounted in paraffin. Sections (5μm
5   thick) were cut through the calvaria adjacent to the
lambdoid suture and stained with either hematoxylin and
eosin or reacted for tartrate resistant acid
phosphatase activity (Sigma Kit# 387A) and
counterstained with hematoxylin. Bone resorption was
10   assessed in the IL-1 α treated mice by
histomorphometric methods using the Osteomeasure
(Osteometrics, Atlanta, GA) by tracing histologic
features onto a digitizor platen using a microscope-
mounted camera lucida attachment. Osteoclast numbers,
15   osteoclast lined surfaces, and eroded surfaces were
determined in the marrow spaces of the calvarial bone.
The injected and non-injected sides of the calvaria
were measured separately.

Results

20   IL-1 α and IL-1 β produced hypercalcemia at the
doses used, particularly on the second day, presumably
by the induction of increased bone resorption
systemically. The hypercalcemic response was blocked by
muOPG [22-401]-Fc in the IL-1 beta treated mice and
25   significantly diminished in mice treated with IL-1-α,
an effect most apparent on day 2 (Figure 22A-22B).

Histologic analysis of the calvariae of mice
treated with IL-1-α and beta shows that IL-1 treatments
alone produce a marked increase in the indices of bone
30   resorption including: osteoclast number, osteoclast
lined surface, and eroded surface (surfaces showing
deep scalloping due to osteoclastic action (Figure
23B). In response to IL-1 α or IL-1 β, the increases in
bone resorption were similar on the injected and non-

injected sides of the calvaria. Muopg [22-401]-Fc
injections reduced bone resorption in both IL-1-α and
beta treated mice and in mice receiving vehicle alone
but this reduction was seen only on the muopg [22-401]-
5   Fc injected sides of the calvariae.

      The most likely explanation for these observations
is that muOPG [22-401]-Fc inhibited bone resorption, a
conclusion supported by the reduction of both the total
osteoclast number and the percentage of available bone
10   surface undergoing bone resorption, in the region of
the calvaria adjacent to the muOPG [22-401]-Fc
injection sites. The actions of muOPG [22-401]-Fc
appeared to be most marked locally by histology, but
the fact that muOPG [22-401]-Fc also blunted IL-1
15   induced hypercalcemia suggests that muOPG [22-401]-Fc
has more subtle effects on bone resorption
systemically.

C. Systemic Effects of muOPG [22-401]-Fc in Growing
Mice

20   Male BDF1 mice aged 3-4 weeks, weight range 9.2-
15.7g were divided into groups of ten mice per group.
These mice were injected subcutaneously with saline or
muOPG [22-401]-Fc 2.5mg/kg bid for 14 days
(5mg/kg/day). The mice were radiographed before
25   treatment, at day 7 and on day 14. The mice were
sacrificed 24 hours after the final injection. The
right femur was removed, fixed in zinc formalin,
decalcified in formic acid and embedded in paraffin.
Sections were cut through the mid region of the distal
30   femoral metaphysis and the femoral shaft. Bone density,
by histomorphometry, was determined in six adjacent
regions extending from the metaphyseal limit of the
growth plate, through the primary and secondary
spongiosa and into the femoral diaphysis (shaft). Each
35   region was 0.5 X 0.5 mm^2.

Radiographic changes

After seven days of treatment there was evidence
of a zone of increased bone density in the spongiosa
associated with the growth plates in the OPG treated
5   mice relative to that seen in the controls. The effects
were particularly striking in the distal femoral and
the proximal tibial metaphases (Figure 24A-24B).
However bands of increased density were also apparent
in the vertebral bodies, the iliac crest and the distal
10   tibia. At 14 days, the regions of opacity had extended
further into the femoral and tibial shafts though the
intensity of the radio-opacity was diminished.
Additionally, there were no differences in the length
of the femurs at the completion of the experiment or in
15   the change in length over the duration of the
experiment implying that OPG does not alter bone
growth.

Histological Changes

The distal femoral metaphysis showed increased
20   bone density in a regions 1.1 to 2.65 mm in distance
from the growth plate (Figures 25 and 26A-26B). This is
a region where bone is rapidly removed by osteoclast-
mediated bone resorption in mice. In these rapidly
growing young mice, the increase in bone in this region
25   observed with OPG treatment is consistent with an
inhibition of bone resorption.

D. Effects of Osteoprotegerin on Bone Loss Induced by
Ovariectomy in the Rat

Twelve week old female Fisher rats were
30   ovariectomized (OVX) or sham operated and dual xray
absorptiometry (DEXA) measurements made of the bone
density in the distal femoral metaphysis. After 3 days
recovery period, the animals received daily injections
for 14 days as follows: Ten sham operated animals
35   received vehicle (phosphate buffered saline); Ten OVX
animals received vehicle (phosphate buffered saline);

Six OVX animals received OPG-Fc 5mg/kg SC; Six OVX
animals received pamidronate (PAM) 5mg/kg SC; Six OVX
animals received estrogen (ESTR) 40ug/kg SC. After 7
and 14 days treatment the animals had bone density
5   measured by DEXA. Two days after the last injection the
animals were killed and the right tibia and femur
removed for histological evaluation.

The DEXA measurements of bone density showed a
trend to reduction in the bone density following
10   ovariectomy that was blocked by OPG-Fc. Its effects
were similar to the known antiresorptive agents
estrogen and pamidronate. (Figure 27). The
histomorphometric analysis confirmed these observations
with OPG-Fc treatment producing a bone density that was
15   significantly higher in OVX rats than that seen in
untreated OVX rats (Figure 28). These results confirm
the activity of OPG in the bone loss associated with
withdrawal of endogenous estrogen following
ovariectomy.

20   In vivo Summary
The in vivo actions of recombinant OPG parallel
the changes seen in OPG transgenic mice. The reduction
in osteoclast number seen in the OPG transgenic is
reproduced by injecting recombinant OPG locally over
25   the calvaria in both normal mice and in mice treated
with IL-1 α or IL-1 β. The OPG transgenic mice develop
an osteopetrotic phenotype with progressive filling of
the marrow cavity with bone and unremodelled cartilage
extending from the growth plates from day 1 onward
30   after birth. In normal three week old (growing) mice,
OPG treatments also led to retention of bone and
unremodelled cartilage in regions of endochondral bone
formation, an effect observed radiographically and
confirmed histologically. Thus, recombinant OPG
35   produces phenotypic changes in normal animals similar

to those seen in the transgenic animals and the changes
are consistent with OPG-induced inhibition of bone
resorption. Based on in vitro assays of osteoclast
formation, a significant portion of this inhibition is

5   due to impaired osteoclast formation. Consistent with
this hypothesis, OPG blocks ovariectomy-induced
osteoporosis in rat. Bone loss in this model is known
to be mediated by activated osteoclasts, suggesting a
role for OPG in treatment of primary osteoporosis.

10   **EXAMPLE 12**
            Pegylation Derivatives of OPG
    Preparation of N-terminal PEG-OPG conjugates by
    reductive alkylation
        HuOPG met [22-194] P25A was buffer exchanged into

15 25-50 mM NaOAc, pH 4.5-4.8 and concentrated to 2-5
    mg/ml. This solution was used to conduct OPG reductive
    alkylation with monofunctional PEG aldehydes at 5-7 C.
    PEG monofunctional aldehydes, linear or branched, MW=1
    to 57 kDa (available from Shearwater Polymers) were

20 added to the OPG solution as solids in amounts
    constituting 2-4 moles of PEG aldehyde per mole of OPG.
    After dissolution of polymer into the protein solution,
    sodium cyanoborohydride was added to give a final
    concentration of 15 to 20 mM in the reaction mixture

25 from 1-1.6 M freshly prepared stock solution in cold DI
    water. The progress of the reaction and the extent of
    OPG PEGylation was monitored by size exclusion HPLC on
    a G3000SW$_{XL}$ column (Toso Haas) eluting with 100 mM
    NaPO$_4$, 0.5 M NaCl, 10% ethanol, pH 6.9. Typically the

30 reaction was allowed to proceed for 16-18 hours, after
    which the reaction mixture was diluted 6-8 times and
    the pH lowered to 3.5-4. The reaction mixture was
    fractionated by ion exchange chromatography (HP SP
    HiLoad 16/10, Pharmacia) eluting with 20 mM NaOAc pH 4

35 with a linear gradient to 0.75M NaCl over 25 column

volumes at a flow rate of 30 cm/h. Fractions of mono-,
di- or poly- PEGylated OPG were pooled and
characterized by SEC HPLC and SDS-PAGE. By N-terminal
sequencing, it was determined that the monoPEG-OPG

5   conjugate, the major reaction product in most cases,
was 98% N-terminally PEG-modified OPG.

This procedure was generally used to prepare the
following N-terminal PEG-OPG conjugates (where OPG is
HuOPG met [22-194] P25A: 5 kD monoPEG, 10 kD mono

10 branched PEG, 12 kD monoPEG, 20 kD monoPEG, 20 kD mono
branched PEG, 25 kD monoPEG, 31 kD monoPEG, 57 kD
monoPEG, 12 kD diPEG, 25 kD diPEG, 31 kD diPEG, 57 kD
diPEG, 25 kD triPEG.

Preparation of PEG-OPG conjugates by acylation

15   HuOPG met [22-194] P25A was buffer exchanged into
50 mM BICINE buffer, pH 8 and concentrated to 2-3
mg/ml. This solution was used to conduct OPG acylation
with monofunctional PEG N-hydroxysuccinimidyl esters at
room temperature. PEG N-hydroxysuccinimidyl esters,

20 linear or branched, MW=1 to 57 kDa (available from
Shearwater Polymers) were added to the OPG solution as
solids in amounts constituting 4-8 moles of PEG N-
hydroxysuccinimidyl ester per mole of OPG. The progress
of the reaction and the extent of OPG PEGylation was

25 monitored by size exclusion HPLC on a G3000SW$_{XL}$ column
(Toso Haas) eluting with 100 mM NaPO$_4$, 0.5 M NaCl, 10%
ethanol, pH 6.9. Typically the reaction was allowed to
proceed for 1 hour, after which the reaction mixture
was diluted 6-8 times and the pH lowered to 3.5-4. The

30 reaction mixture was fractionated by ion exchange
chromatography (HP SP HiLoad 16/10, Pharmacia) eluting
with 20 mM NaOAc pH 4 with a linear gradient to 0.75M
NaCl over 25 column volumes at a flow rate of 30 cm/h.
Fractions of mono-, di- or poly- PEGylated OPG were

35 pooled and characterized by SEC HPLC and SDS-PAGE.

This procedure was generally used to prepare the following PEG-OPG conjugates: 5 kD polyPEG, 20 kD polyPEG, 40 kD poly branched PEG, 50 kD poly PEG.

Preparation of dimeric PEG-OPG

5   HuOPG met [22-194] P25A is prepared for thiolation at 1-3 mg/ml in a phosphate buffer at near neutral pH. S-acetyl mecaptosuccinic anhydride (AMSA) is added in a 3-7 fold molar excess while maintaining pH at 7.0 and the rxn stirred for ·2 hrs. The monothiolated-OPG

10   is separated from unmodified and polythiolated OPG by ion exchange chromatography and the protected thiol deprotected by treatment with hydroxylamine. After deprotection, the hydroxylamine is removed by gel filtration and the resultant monothiolated-OPG is

15   subjected to a variety of thiol specific crosslinking chemistries. To generate a disulfide bonded dimer, the thiolated OPG at >1mg/ml is allowed to undergo air oxidation by dialysis in slightly basic phosphate buffer. The covalent thioether OPG dimer was prepared

20   by reacting the bis-maleimide crosslinker, N,N-bis(3-maleimido propianyl)-2-hydroxy 1,3 propane with the thiolated OPG at >1mg/ml at a 0.6x molar ratio of crosslinker:OPG in phosphate buffer at pH 6.5. Similarly, the PEG dumbbells are produced by reaction

25   of substoichiometric amounts of bis-maleimide PEG crosslinkers with thiolated OPG at >1mg/ml in phosphate buffer at pH 6.5. Any of the above dimeric conjugates may be further purified using either ion exchange or size exclusion chromatographies.

30   Dimeric PEG-OPG conjugates (where OPG is HuOPG met [22-194] P25A prepared using the above procedures include disulfide-bonded OPG dimer, covalent thioether OPG dimer with an aliphatic amine type crosslinker, 3.4 kD and 8kD PEG dumbbells and monobells.

PEG-OPG conjugates were tested for activity in vitro using the osteoclast maturation assay described in Example 11A and for activity in vivo by measuring increased bone density after injection into mice as described in Example 11C. The in vivo activity is shown below in Table 2.

Table 2

In vivo biological activity of Pegylated OPG

OPG Construct	Increase in Tibial Bone Density
muOPG met [22-194]	-
muOPG met [22-194] 5k PEG	+
muOPG met [22-194] 20k PEG	+
huOPG met [22-194] P25A	-
huOPG met [22-194] P25A 5k PEG	+
huOPG met [22-194] P25A 20k PEG	+
huOPG met [22-194] P25A 31k PEG	+
huOPG met [22-194] P25A 57k PEG	+
huOPG met [22-194] P25A 12k PEG	+
huOPG met [22-194] P25A 20k Branched PEG	+
huOPG met [22-194] P25A 8k PEG dimer	+
huOPG met [22-194] P25A disulfide crosslink	+

EXAMPLE 13

Effects of OPG-Fc during the course of Adjuvant Arthritis in Lewis rats

The aim of these studies is to investigate whether CHO produced OPG-Fc protects against adjuvant arthritis-associated bone mineral density loss in male Lewis rats.

Animals

Male Lewis rats (Charles River, Wilmington MA) 8-9 weeks of age (n = 6) at the time of mycobacteria in oil injection, were used. Two rats were housed per cage in an air conditioned environment (room temperature 23 $\pm$ 2

165

C, relative humidity 50 ± 20%) that illuminated from
6:30 am to 6:30 p.m. Animals were fed a commercial
rodent chow (#8640, Tek Lab, Madison WI); calcium and
phosphorus contents were 1.2% and 1.0%, respectively.
5   All animals were sacrificed by carbon dioxide
inhalation.

Induction and Measurement of Adjuvant Arthritis

    Adjuvant arthritis (AdA) was induced by a single
injection of a suspension of Mycobacterium tuberculosis
10 (Difco Laboratories, Detroit MI) in paraffin oil
(Crescent Chemical Co., Hauppauge, NY). Mycobacteria
were grounded in a mortar to fine powder and suspended
in paraffin oil (10 mg/ml). The suspension was
dispersed evenly just before injection of 0.05ml at the
15 base of tail. Severity of inflammation was monitored by
measuring the volume of hindpaws using volume
displacement technique. The extent of inflammation was
calculated as increase in paw volume compared to Day 0.
In addition, body weight was measured daily.

20 OPG treatment and DEXA bone mass measurement

    Male Lewis (normal and adjuvant-induced) rats
received varying doses of OPG-Fc (22-194) by
subcutaneous daily injection (See graphs below for
dosing) from day 9 to day 15. At the end of the
25 experiment (day 16) bone mass measurement (DexaScans)
of the tibiotarsal region was performed with a Hologic
QDR 4500 dual-energy x-ray absorptiometer.

Statistical Analysis

    All results were expressed as the mean ± standard
30 deviation of the mean. The p value of 0.05 was used in
the calculation to determine whether there were any
significant differences between any two groups.
Statistical significance of difference was assessed by
analysis of variance based on a Mann Whitney U test
35 using Statsoft software (Statsoft, Tulsa, OK).

Results

OPG-Fc inhibits loss of Bone Mineral Density in
adjuvant arthritis

To study the effects of OPG-Fc on BMD in adjuvant
5   arthritis, paws from two experiments were analyzed by
DEXA. The results of BMD measurements on the
tibiotarsal region are shown in Figures 2 and 4. Bone
protective effects were observed in rats with adjuvant-
arthritis treated with OPG-Fc via subcutaneous daily
10   injection (from day 9 to day 15 after mycobacteria
injection). Treatment with OPG-Fc at 4, 1, 0.25, 0.06,
.016, and 0.004 mg/kg showed 100%, 100%, 100%, 86%, 22,
and 22% inhibition of bone mineral density loss
respectively. Treatment of the intermediate and high
15   doses of OPG-Fc (4 - 0.06 mg/kg) showed a statistically
significant difference in BMD when compared to the OPG
placebo treated control group (P < 0.05).

However, treatment with OPG-Fc (at all doses) had
no statistically significant effect on the severity of
20   inflammation (Figure 1 and 3, AUC) or loss of body
weight (data on file).

Conclusion

In conclusion, the results demonstrate that OPG-Fc
have great efficacy in preventing bone density loss in
25   the tibiotarsal region in arthritic rats. The
inhibitory effects of OPG-Fc against bone changes
occurred without any anti-inflammatory actions.

EXAMPLE 14

Combination treatment with OPG-Fc and sTNF-RI on
Adjuvant Arthritis in Male Lewis Rats

30   Male Lewis rats were injected with 0.5 mg heat-
killed Mycobacterium tuberculosis H37Ra in mineral oil
at the base of the tail. Rats were monitored for paw
swelling and weight loss. Arthritis (paw swelling)
35   developed after about 10 days. Paw swelling was
calculated daily relative to paw volume on day 9

(beginning of treatment) and the area under the curve (AUC) from day 9 to 15 is given in the graph (Figure 31A). On day 16 at the end of the experiment DexaScans of the rats were taken and the calcaneus was evaluated

5   for loss of bone mineral density (BMD) as shown in Figure 31B.

                    *   *   *

     While the invention has been described in what is considered to be its preferred embodiments, it is not

10   to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such

15   modifications and equivalents.

Claims

What is claimed is:

1.   A method for treating conditions leading to bone
     loss, which comprises administering a
     therapeutically effective amount of a purified and
     isolated OPG protein in conjunction with
     substances selected from the group consisting of
     TNF-α inhibitors; serine protease inhibitors; IL-1
     inhibitors; IL-6 inhibitors; IL-8 inhibitors; IL-
     18 inhibitors; ICE modulators; FGF-1 to FGF-10;
     FGF modulators; PAF antagonists; MMP modulators;
     NOS modulators; modulators of glucocorticoid
     receptor; modulators of glutamate receptor;
     modulators of LPS levels; and noradrenaline and
     modulators and mimetics thereof.

2.   The method of Claim 1, wherein the OPG protein is
     OPG-Fc.

3.   The method of claim 1, wherein an IL-1 inhibitor
     and the OPG protein are administered.

4.   The method of claim 3, wherein the IL-1 inhibitor
     comprises an IL-1ra polypeptide.

5.   The method of Claim 4, wherein the OPG protein
     comprises an Fc region.

6.   The method of claim 1, wherein a TNF-α inhibitor
     and the OPG protein are administered.

7.   The method of claim 6, wherein the TNF-α inhibitor
     comprises sTNFR-I, sTNFR-II, or a fragment of
     sTNF-RI or sTNF-RII linked to an Fc region.

8.   The method of claim 6, wherein the TNF-α inhibitor
     comprises 30 kD PEG sTNFR-I.

9.   The method of claim 6, wherein the TNF-α inhibitor
     comprises a 2.6 kD sTNF-RI fragment.

10. The method of claim 9, wherein the sTNF-RI
     fragment comprises 30 kD PEG.

11. The method of claim 6, wherein the TNF-α inhibitor comprises sTNF-RII linked to an Fc region.

12. The method of claim 6, wherein the TNF-α inhibitor is etanercept.

13. The method of Claim 10, wherein the OPG protein is OPG-Fc.

14. The method of claim 1, wherein a serine protease inhibitor and the OPG protein are administered.

15. The method of claim 14, wherein the serine protease inhibitor comprises a SLPI polypeptide.

16. The method of Claim 14, wherein the OPG protein is OPG-Fc.

17. The method of claim 1, wherein an IL-1 inhibitor, a TNF-α inhibitor, and the OPG protein are administered.

18. The method of claim 17, wherein the IL-1 inhibitor comprises an IL-1ra polypeptide.

19. The method of claim 17, wherein the TNF-α inhibitor comprises sTNFR-I, sTNFR-II, sTNFR fragments or sTNFR-Fc.

20. The method of claim 17, wherein the TNF-α inhibitor comprises 30 kD PEG-sTNFR-I.

21. The method of claim 17, wherein the sTNF-RI fragment is a 2.6 kD fragment.

22. The method of claim 21, wherein the sTNF-RI fragment comprises 30 kD PEG.

23. The method of claim 17, wherein the TNF-α inhibitor comprises sTNFR-II linked to an Fc region.

24. The method of claim 17, wherein the TNF-α inhibitor is etanercept.

25. The method of Claim 17, wherein the OPG protein is OPG-Fc.

26. The method of Claim 1, wherein an IL-1 inhibitor, a serine protease inhibitor, and the OPG protein are administered.

27. The method of claim 26, wherein the IL-1 inhibitor comprises an IL-1ra polypeptide.

28. The method of claim 26, wherein the serine protease inhibitor comprises a SLPI polypeptide.

29. The method of Claim 26, wherein the OPG protein is OPG-Fc.

30. The method of claim 1, wherein a serine protease inhibitor, a TNF-α inhibitor, and the OPG protein are administered.

31. The method of claim 30, wherein the serine protease inhibitor comprises a SLPI polypeptide.

32. The method of claim 17, wherein the TNF-α inhibitor comprises sTNFR-I, sTNFR-II, sTNFR fragments or sTNFR-Fc.

33. The method of claim 30, wherein the TNF-α inhibitor comprises 30 kD PEG sTNFR-I.

34. The method of claim 30, wherein the TNF-α inhibitor comprises sTNFR-II linked to an Fc region.

35. The method of claim 30, wherein the TNF-α inhibitor is etanercept.

36. The method of claim 30, wherein the TNF-α inhibitor comprises a 2.6 kD sTNF-RI fragment.

37. The method of claim 36, wherein the sTNF-RI fragment comprises 30 kD PEG.

38. The method of Claim 30, wherein the OPG protein is OPG-Fc.

39. The method of any of claims 17 to 38, wherein the condition treated is rheumatoid arthritis.

40. The method of any of claims 17 to 38, wherein the condition treated is multiple sclerosis.

41. The method of any of claims 17 to 38, wherein the condition treated is osteoporosis.

42. The method of any of claims 17 to 38, wherein the condition treated is osteomyelitis.

43. A method of treating an IL-1 mediated disease, which comprises administering therapeutically effective amounts of an IL-1 inhibitor and a serine protease inhibitor.

44. The method of claim 43, wherein the IL-1 inhibitor comprises an IL-1ra polypeptide.

45. The method of claim 43, wherein the serine protease inhibitor comprises a SLPI polypeptide.

46. The method of claim 43, wherein the IL-1 mediated disease is asthma.

47. The method of claim 43, wherein the IL-1 mediated disease is rheumatoid arthritis.

48. The method of claim 46, wherein the therapeutically effective amounts are delivered by pulmonary administration.

49. A method of treating TNF-mediated disease, which comprises administering therapeutically effective amounts of a TNF-α inhibitor and a serine protease inhibitor.

50. The method of claim 49, wherein the TNF-α inhibitor comprises sTNFR-I, sTNFR-II, sTNFR fragments or sTNFR-Fc.

51. The method of claim 49, wherein the TNF-α inhibitor comprises 30 kD PEG sTNFR-I.

52. The method of claim 49, wherein the TNF-α inhibitor comprises a 2.6 kD sTNF-RI fragment.

53. The method of claim 52, wherein the sTNF-RI fragment comprises 30 kD PEG.

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54. The method of claim 49, wherein the TNF-α inhibitor comprises sTNFR-II linked to an Fc region.

55. The method of claim 49, wherein the TNF-α inhibitor is etanercept.

56. The method of claim 49, wherein the serine protease inhibitor comprises a SLPI polypeptide.

57. The method of claim 49, wherein the TNF-mediated disease is rheumatoid arthritis.

58. A method of treating inflammation, which comprises administering an IL-18 inhibitor, a TNF-α inhibitor, and an IL-1 inhibitor.

59. A method of treating rheumatoid arthritis, which comprises administering an IL-18 inhibitor, a TNF-α inhibitor, and an IL-1 inhibitor.

60. A method of treating SLE, which comprises administering an IL-18 inhibitor, a TNF-α inhibitor, and an IL-1 inhibitor.

61. A method of treating GvHD, which comprises administering an IL-18 inhibitor, a TNF-α inhibitor, and an IL-1 inhibitor.

FIG.1A

```
              148        178       208       238       268       298
FRI-1         ALLVFLDIIEWTTQETFPPKYLHYDPETGRQLLCDKCAPGTYLKQHCTVRRKTLCVPCPD
                          |:| | |:||  |   |   ||   | || | |:| :| |
SW:TNR2_HUMAN HALPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCED
                    30        40        50        60        70        80

              328
FRI-1         YSYTDSWHTS
              :||: |::
SW:TNR2_HUMAN STYTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPL
                    90       100       110       120       130       140
```

FIG.1B

```
FRI-1         69 YLHYDPETGRQLLCDKCAPGTYLKQHC.TVRRKTLCV.PCPDY.SYTDSW
                  | |.  .:.  | .|||:||:.| .|  .| |:  ||.:  |.|.
TNFR profile   6 YHYDQNGRMCEECHMCQPGHFLVKHCKQPKRDTVCHKPCEPGVTYTDDW

FRI-1        116 H
                 |
TNFR profile  56 H                                Z Score = 8.29
```

FIG. IC

FIG.2A

AUG   TAG

SP

FIG.2B

```
        10                    30                    50
ATCAAAGGCAGGGCATACTTCCTGTTGCCCAGACCTTATATAAAACGTCATGTTCGCCTG
        70                    90                   110
GGCAGCAGAGAAGCACCTAGCACTGGCCCAGCGGCTGCCGCCTGAGGTTTCCAGAGGACC
       130                   150                   170
ACAATGAACAAGTGGCTGTGCTGTGCACTCCTGGTGTTCTTGGACATCATTGAATGGACA
        M  N  K  W  L  C  C  A  L  L  V  F  L  D  I  I  E  W  T
       190                   210                   230
ACCCAGGAAACCTTTCCTCCAAAATACTTGCATTATGACCCAGAAACCGGACGTCAGCTC
 T  Q  E  T  F  P  P  K  Y  L  H  Y  D  P  E  T  G  R  Q  L
       250                   270                   290
TTGTGTGACAAATGTGCTCCTGGCACCTACCTAAAACAGCACTGCACAGTCAGGAGGAAG
 L  C  D  K  C  A  P  G  T  Y  L  K  Q  H  C  T  V  R  R  K
       310                   330                   350
ACACTGTGTGTCCCTTGCCCTGACTACTCTTATACAGACAGCTGGCACACGAGTGATGAA
 T  L  C  V  P  C  P  D  Y  S  Y  T  D  S  W  H  T  S  D  E
       370                   390                   410
TGCGTGTACTGCAGCCCCGTGTGCAAGGAACTGCAGACCGTGAAACAGGAGTGCAACCGC
 C  V  Y  C  S  P  V  C  K  E  L  Q  T  V  K  Q  E  C  N  R
       430                   450                   470
ACCCACAACCGAGTGTGCGAATGTGAGGAAGGGCGCTACTGGAGCTCGAATTCTGCTTG
 T  H  N  R  V  C  E  C  E  E  G  R  Y  L  E  L  E  F  C  L
       490                   510                   530
AAGCACCGGAGCTGTCCCCCAGGCTTGGGTGTGCTGCAGGCTGGGACCCCAGAGCGAAAC
 K  H  R  S  C  P  P  G  L  G  V  L  Q  A  G  T  P  E  R  N
       550                   570                   590
ACGGTTTGCAAAAGATGTCCGGATGGGTTCTTCTCAGGTGAGACGTCATCGAAAGCACCC
 T  V  C  K  R  C  P  D  G  F  F  S  G  E  T  S  S  K  A  P
       610                   630                   650
TGTAGGAAACACACCAACTGCAGCTCACTTGGCCTCCTGCTAATTCAGAAAGGAAATGCA
 C  R  K  H  T  N  C  S  S  L  G  L  L  L  I  Q  K  G  N  A
       670                   690                   710
ACACATGACAATGTATGTTCCGGAAACAGAGAAGCAACTCAAAATTGTGGAATAGATGTC
 T  H  D  N  V  C  S  G  N  R  E  A  T  Q  N  C  G  I  D  V
       730                   750                   770
ACCCTGTGCGAAGAGGCATTCTTCAGGTTTGCTGTGCCTACCAAGATTATACCGAATTGG
 T  L  C  E  E  A  F  F  R  F  A  V  P  T  K  I  I  P  N  W
       790                   810                   830
CTGAGTGTTCTGGTGGACAGTTTGCCTGGGACCAAAGTGAATGCAGAGAGTGTAGAGAGG
 L  S  V  L  V  D  S  L  P  G  T  K  V  N  A  E  S  V  E  R
       850                   870                   890
ATAAAAACGGAGACACAGCTCGCAAGAGCAAACTTTCCAGCTACTTAAGCTGTGGAAGCAT
 I  K  R  R  H  S  S  Q  E  Q  T  F  Q  L  L  K  L  W  K  H
       910                   930                   950
CAAAACAGAGACCAGGAAATGGTGAAGAAGATCATCCAAGACATTGACCTCTGTGAAAGC
 Q  N  R  D  Q  E  M  V  K  K  I  I  Q  D  I  D  L  C  E  S
       970                   990                  1010
AGTGTGCAACGGCATATCGGCCACGCGAACCTCACCACAGAGCAGCTCCGCATCTTGATG
 S  V  Q  R  H  I  G  H  A  N  L  T  T  E  Q  L  R  I  L  M
```

FIG.2C

```
       1030              1050                    1070
GAGAGCTTGCCTGGGAAGAAGATCAGCCCAGACGAGATTGAGAGAACGAGAAAGACCTGC
E   S   L   P   G   K   K   I   S   P   D   E   I   E   R   T   R   K   T   C
       1090              1110                    1130
AAACCCAGCGAGCAGCTCCTGAAGCTACTGAGCTTGTGGAGGATCAAAAATGGAGACCAA
K   P   S   E   Q   L   L   K   L   L   S   L   W   R   I   K   N   G   D   Q
       1150              1170                    1190
GACACCTTGAAGGGCCTGATGTACGCACTCAAGCACTTGAAAGCATACCACTTTCCCAAA
D   T   L   K   G   L   M   Y   A   L   K   H   L   K   A   Y   H   F   P   K
       1210              1230                    1250
ACCGTCACCCACAGTCTGAGGAAGACCATCAGGTTCTTGCACAGCTTCACCATGTACCGA
T   V   T   H   S   L   R   K   T   I   R   F   L   H   S   F   T   M   Y   R
       1270              1290                    1310
TTGTATCAGAAACTCTTTCTAGAAATGATAGGGAATCAGGTTCAATCAGTGAAGATAAGC
L   Y   Q   K   L   F   L   E   M   I   G   N   Q   V   Q   S   V   K   I   S
       1330              1350                    1370
TGCTTATAGTTAGGAATGGTCACTGGGCTGTTTCTTCAGGATGGGCCAACACTGATGGAG
C   L
       1390              1410                    1430
CAGATGGCTGCTTCTCCGGCTCTTGAAATGGCAGTTGATTCCTTTCTCATCAGTTGGTGG
       1450              1470                    1490
GAATGAAGATCCTCCAGCCCAACACACACACTGGGGAGTCTGAGTCAGGAGAGTGAGGCA
       1510              1530                    1550
GGCTATTTGATAATTGTGCAAAGCTGCCAGGTGTACACCTAGAAAGTCAAGCACCCTGAG
       1570              1590                    1610
AAAGAGGATATTTTTATAACCTCAAACATAGGCCCTTTCCTTCCTCTCCTTATGGATGAG
       1630              1650                    1670
TACTCAGAAGGCTTCTACTATCTTCTGTGTCATCCCTAGATGAAGGCCTCTTTTATTTAT
       1690              1710                    1730
TTTTTTATTCTTTTTTTCGGAGCTGGGGACCGAACCCAGGGCCTTGCGCTTGCGAGGCAA
       1750              1770                    1790
GTGCTCTACCACTGAGCTAAATCTCCAACCCCTGAAGGCCTCTTTCTTTCTGCCTCTGAT
       1810              1830                    1850
AGTCTATGACATTCTTTTTTCTACAATTCGTATCAGGTGCACGAGCCTTATCCCATTTGT
       1870              1890                    1910
AGGTTTCTAGGCAAGTTGACCGTTAGCTATTTTTCCCTCTGAAGATTTGATTCGAGTTGC
       1930              1950                    1970
AGACTTGGCTAGACAAGCAGGGGTAGGTTATGGTAGTTTATTTAACAGACTGCCACCAGG
       1990              2010                    2030
AGTCCAGTGTTTCTTGTTCCTCTGTAGTTGTACCTAAGCTGACTCCAAGTACATTTAGTA
       2050              2070                    2090
TGAAAAATAATCAACAAATTTTATTCCTTCTATCAACATTGGCTAGCTTTGTTTCAGGGC
       2110              2130                    2150
ACTAAAAGAAACTACTATATGGAGAAAGAATTGATATTGCCCCCAACGTTCAACAACCCA
       2170              2190                    2210
ATAGTTTATCCAGCTGTCATGCCTGGTTCAGTGTCTACTGACTATGCGCCCTCTTATTAC
       2230              2250                    2270
TGCATGCAGTAATTCAACTGGAAATAGTAATAATAATAGAAATAAAATCTAGACTCC
       2290              2310                    2330
ATTGGATCTCTCTGAATATGGGAATATCTAACTTAAGAAGCTTTGAGATTTCAGTTGTGT
       2350              2370                    2390
TAAAGGCTTTTATTAAAAAGCTGATGCTCTTCTGTAAAAGTTACTAATATATCTGTAAGA
       2410              2430
```

FIG.2D

FIG.2E

```
                                                      152
                                                      191
                                                      129
                                                      143
                                                      125
                                                      124
                                                      128
                                                      116
                                                      105

                                                      187
                                                      230
                                                      178
                                                      193
                                                      175
                                                      174
                                                      178
                                                      152
                                                      147

                                                      219
                                                      280
                                                      207
                                                      227
                                                      197
                                                      208
                                                      224
                                                      202
                                                      191
```

fas.frg
tnfrl.frg
sfv-t2.frg
tnf2.frg
cd40.frg
osteo.frg
ngfr.frg
ox40.frg
41bb.frg

FIG.3A

FIG.3B

FIG.3C

FIG.3D

FIG.3E

FIG. 3F COOH End

FIG. 3G Turn

FIG. 3H Hydrophobic Moment

FIG. 3I Goldman et al Kyte-Doolittle

FIG.4A

FIG.4B

FIG.5

2 11 16 17 22 28 33 38 45 ⌐Kb⌐ 1 12 18 30

Transgenic Founders   Controls
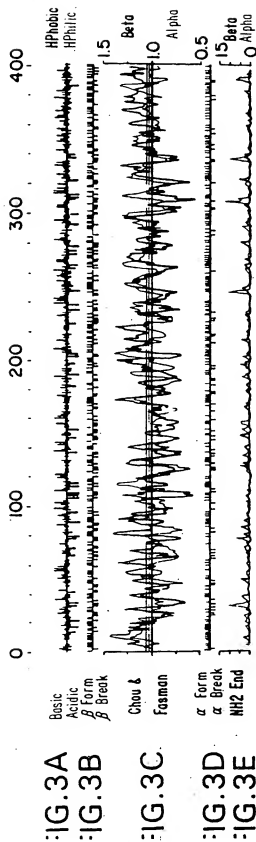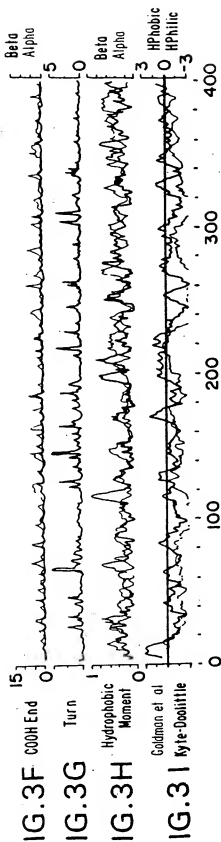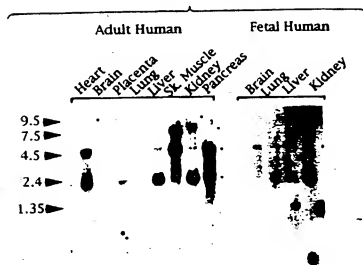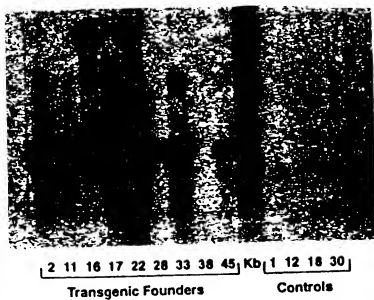
FIG.6A

FIG.6B
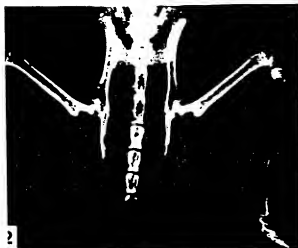
FIG.6C
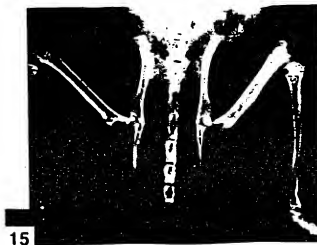
FIG.6D
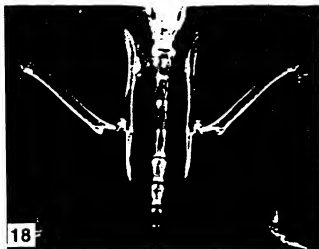
FIG.6E

FIG.6F
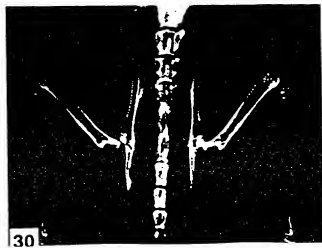
FIG.6G

FIG.6H

FIG.6I

FIG.6J

FIG.7A

FIG.7B

FIG.7C
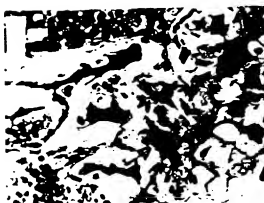
FIG.7D

FIG.7E

FIG.7F
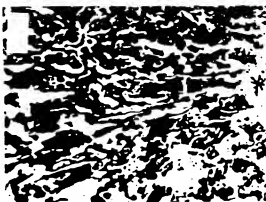
FIG.7G
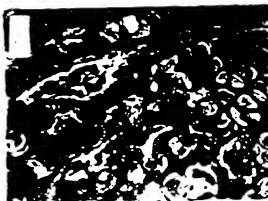
FIG.7H
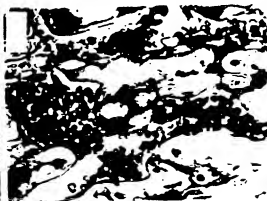
FIG.8A

FIG.8B

FIG.8C

FIG.8D
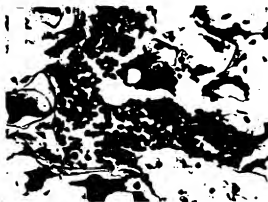
FIG.9A

```
          10                    30                      50
CCTTATATAARACGTCATGATTGCCTGGGCTGCAGAGACGCACCTAGCACTGACCCAGCG
            70                  90                     110
GCTGCCTCCTGAGGTTTCCCGAGGACCACAATGAACAAGTGGCTGTGCTGCGCACTCCTG
                                     M  N  K  W  L  C  C  A  L  L
          130                  150                     170
GTGCTCCTGGACATCATTGAATGGACAACCCAGGAAACCCTTCCTCCAAAGTACTTGCAT
V  L  L  D  I  I  E  W  T  T  Q  E  T  L  P  P  K  Y  L  H
          190                  210                     230
TATGACCCAGAAACTGGTCATCAGCTCCTGTGTGACAAATGTGCTCCTGGCACCTACCTA
Y  D  P  E  T  G  H  Q  L  L  C  D  K  C  A  P  G  T  Y  L
          250                  270                     290
AAACAGCACTGCACAGTGAGGAGGAAGACATTGTGTGTCCCTTGCCCTGACCACTCTTAT
K  Q  H  C  T  V  R  R  K  T  L  C  V  P  C  P  D  H  S  Y
          310                  330                     350
ACGGACAGCTGGCACACCAGTGATGAGTGTGTGTATTGCAGCCCAGTGTGCAAGGAACTG
T  D  S  W  H  T  S  D  E  C  V  Y  C  S  P  V  C  K  E  L
          370                  390                     410
CAGTCCGTGAAGCAGGAGTGCAACCGCACCCACAACCGAGTGTGTGAGTGTGAGGAAGGG
Q  S  V  K  Q  E  C  N  R  T  H  N  R  V  C  E  C  E  E  G
          430                  450                     470
CGTTACCTGGAGATCGAATTCTGCTTGAAGCACCGGAGCTGTCCCCCGGGCTCCGGCGTG
R  Y  L  E  I  E  F  C  L  K  H  R  S  C  P  P  G  S  G  V
          490                  510                     530
GTGCAAGCTGGAACCCCAGAGCGAAACACAGTTTGCAAAAAATGTCCAGATGGGTTCTTC
V  Q  A  G  T  P  E  R  N  T  V  C  K  K  C  P  D  G  F  F
          550                  570                     590
TCAGGTGAGACTTCATCGAAAGCACCCTGTATAAAACACACGAACTGCAGCACATTTGGC
S  G  E  T  S  S  K  A  P  C  I  K  H  T  N  C  S  T  F  G
          610                  630                     650
CTCCTGCTAATTCAGAAAGGAAATGCAACACATGACAACGTGTGTTCCGGAAACAGAGAA
L  L  L  I  Q  K  G  N  A  T  H  D  N  V  C  S  G  N  R  E
          670                  690                     710
GCCACGCAAAAGTGTGGAATAGATGTCACCCTGTGTGAAGAGGCCTTCTTCAGGTTTGCT
A  T  Q  K  C  G  I  D  V  T  L  C  E  E  A  F  F  R  F  A
          730                  750                     770
GTTCCTACCAAGATTATACCAAATTGGCTGAGTGTTTTGGTGGACAGTTTGCCTGGGACC
V  P  T  K  I  I  P  N  W  L  S  V  L  V  D  S  L  P  G  T
```

FIG.9B

```
         790                   810                   830
AAAGTGAATGCCGAGAGTGTAGAGAGGATAAAACGGAGACACAGCTCACAAGAGCAAACC
K  V  N  A  E  S  V  E  R  I  K  R  R  H  S  S  Q  E  Q  T
         850                   870                   890
TTCCAGCTGCTGAAGCTGTGGAAACATCAAAACAGAGACCAGGAAATGGTGAAGAAGATC
F  Q  L  L  K  L  W  K  H  Q  N  R  D  Q  E  M  V  K  K  I
         910                   930                   950
ATCCAAGACATTGACCTCTGTGAAAGCAGCGGTGCAGCGGCATCTCGGCCACTCGAACCTC
I  Q  D  I  D  L  C  E  S  S  V  Q  R  H  L  G  H  S  N  L
         970                   990                   1010
ACCACAGAGCAGCTTCTTGCCTTGATGGAGAGCCTGCCTGGGAAGAAGATCAGCCCAGAA
T  T  E  Q  L  L  A  L  M  E  S  L  P  G  K  K  I  S  P  E
        1030                  1050                  1070
GAGATTGAGAGAACGAGAAAGACCTGCAAATGCGAGCGAGCAGCTCCTGAAGCTACTCAGT
E  I  E  R  T  R  K  T  C  K  S  S  E  Q  L  L  K  L  L  S
        1090                  1110                  1130
TTATGGAGGATCAAAAATGGTGACCAAGCACACCTTGAAGGGCCTGATGTATGCCCTCAAG
L  W  R  I  K  N  G  D  Q  D  T  L  K  G  L  M  Y  A  L  K
        1150                  1170                  1190
CACTTGAAAACATCCCACTTTCCCAAAACTGTCACCCACAGTCTGAGGAAGACCATGAGG
H  L  K  T  S  H  F  P  K  T  V  T  H  S  L  R  K  T  M  R
        1210                  1230                  1250
TTCCTGCACAGCTTCACAATGTACAGACTGTATCAGAAGCTCTTTTTAGAAATGATAGGG
F  L  H  S  F  T  M  Y  R  L  Y  Q  K  L  F  L  E  M  I  G
        1270                  1290                  1310
AATCAGGTTCAATCCGTGAAAATAAGCTGCTTATAACTAGGAATGGTCACTGGGCTGTTT
N  Q  V  Q  S  V  K  I  S  C  L

CTTCA
```

FIG.9C

```
        10                    30                       50
GTATATATAACGTGATGAGCGTACGGGTGCGGAGACGCACCGGAGCGCTCGCCCAGCCGC
           70                    90                   110
CGYCTCCAAGCCCCTGAGGTTTCCGGGGACCACAATGAACAAGTTGCTGTGCTGCGCGCT
                                          M  N  K  L  L  C  C  A  L
        130                   150                     170
CGTGTTTCTGGACATCTCCATTAAGTGGACCACCCAGGAAACGTTTCCTCCAAAGTACCT
 V  F  L  D  I  S  I  K  W  T  T  Q  E  T  F  P  P  P  K  Y  L
        190                   210                     230
TCATTATGACGAAGAAACCTCTCATCAGCTGTTGTGTGACAAATGTCCTCCTGGTACCTA
 H  Y  D  E  E  T  S  H  Q  L  L  C  D  K  C  P  P  G  T  Y
        250                   270                     290
CCTAAAACAACACTGTACAGCAAAGTGGAAGACCGTGTGCGCCCCTTGCCCTGACCACTA
 L  K  Q  H  C  T  A  K  W  K  T  V  C  A  P  C  P  D  H  Y
        310                   330                     350
CTACACAGACAGCTGGCACACCAGTGACGAGTGTCTATACTGCAGCCCCGTGTGCAAGGA
 Y  T  D  S  W  H  T  S  D  E  C  L  Y  C  S  P  V  C  K  E
        370                   390                     410
GCTGCAGTACGTCAAGCAGGAGTGCAATCGCCACCCACAACCGCGTGTGCGAATGCAAGGA
 L  Q  Y  V  K  Q  E  C  N  R  T  H  N  R  V  C  E  C  K  E
        430                   450                     470
AGGGCGCTACCTTGAGATAGAGTTCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTTGG
 G  R  Y  L  E  I  E  F  C  L  K  H  R  S  C  P  P  G  F  G
        490                   510                     530
AGTGGTGCAAGCTGGAACCCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTT
 V  V  Q  A  G  T  P  E  R  N  T  V  C  K  R  C  P  D  G  F
        550                   570                     590
CTTCTCAAATGAGACGTCATCTAAAGCACCCTGTAGAAAACACACAAATTGCAGTGTCTT
 F  S  N  E  T  S  S  K  A  P  C  R  K  H  T  N  C  S  V  F
        610                   630                     650
TGGTCTCCTGCTAACTCAGAAAGGAAATGCAACACACGACAACATATGTTCCGGAAACAG
 G  L  L  L  T  Q  K  G  N  A  T  H  D  N  I  C  S  G  N  S
        670                   690                     710
TGAATCAACTCAAAAATGTGGAATAGATGTTACCCTGTGTGAGGAGGCATTCTTCAGGTT
 E  S  T  Q  K  C  G  I  D  V  T  L  C  E  E  A  F  F  R  F
        730                   750                     770
TGCTGTTCCTACAAAGTTTACGCCTAACTGGCTTAGTGTCTTGGTAGACAATTTGCCTGG
 A  V  P  T  K  F  T  P  N  W  L  S  V  L  V  D  N  L  P  G
```

FIG.9D

```
       790                    810                        830
CACCAAAGTAAACGCAGAGAGTGTAGAGAGGATAAAACGGCAACACAGCTCACAAGAACA
 T  K  V  N   A  E  S  V   E  R   I  K  R  Q  H  S  S   Q  E  Q
       850                    870                        890
GACTTTCCAGCTGCTGAAGTTATGGAAACATCAAAACAAAGACCAAGATATAGTCAAGAA
 T  F  Q  L   L  K  L  W   K  H  Q  N  K  D  Q   D  I  V  K  K
       910                    930                        950
GATCATCCAAGATATTGACCTCTGTGAAAACAGCGTGCAGCGGCACATTGGACATGCTAA
 I  I  Q  D   I  D  L  C   E  N  S  V  Q  R  H   I  G  H  A  N
       970                    990                       1010
CCTCACCTTCGAGCAGCTTCGTAGCTTGATGGAAAGCTTACCGGGAAAGAAAGTGGGAGC
 L  T  F  E   Q  L  R  S   L  M  E  S  L  P  G   K  K  V  G  A
      1030                   1050                       1070
AGAAGACATTGAAAAAACAATAAAGGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCT
 E  D  I  E   K  T  I  K   A  C  K  P  S  D  Q   I  L  K  L  L
      1090                   1110                       1130
CAGTTTGTGGCGAATAAAAAAATGGCGACCAAGACACCTTGAAGGGCCTAATGCACGCACT
 S  L  W  R   I  K  N  G   D  Q  D  T  L  K  G   L  M  H  A  L
      1150                   1170                       1190
AAAGCACTCAAAGACGTACCACTTTCCCAAAACTGTCACTCAGAGTCTAAAGAAGACCAT
 K  H  S  K   T  Y  H  F   P  K  T  V  T  Q  S   L  K  K  T  I
      1210                   1230                       1250
CAGGTTCCTTCACAGCTTCACAATGTACAAATTGTATCAGAAGTTATTTTTAGAAATGAT
 R  F  L  H   S  F  T  M   Y  K  L  Y  Q  K  L   F  L  E  M  I
      1270                   1290                       1310
AGGTAACCAGGTCCAATCAGTAAAAATAAGCTGCTTATAACTGGAAATGGCCATTGAGCT
 G  N  Q  V   Q  S  V  K   I  S  C  L
      1330                   1350
GTTTCCTCACAATTGGCGAGATCCCATGGATGATAA
```

FIG.9E

```
                                                                          50
muosteo.frg  HNKWLCCALLVLLDIIEWTTQETLPPKYLHYDPETGHQLLCDKCAPGTYL   50
ratosteo.frg HHNKWLCCALLVFLDIIEWTTQETFPPKYLHYDPETGRQLLCDKCAPGTYL  50
huosteo.frg  HNKLLCCALLVFLDISLRWTTQETFPPKYLDETSHQLLCDKCPPGTYL     50

                                                                          100
muosteo.frg  KQHCTVRRKKTLCVPCPDHSYTDSWHTSDECVYCSPVCKELQSVKQECNRT  100
ratosteo.frg KQHCTVRRKKTLCVPCPDYSYTDSWHTSDECVYCSPVCKELQYVKQECNRT  100
huosteo.frg  KQHCTARWKTVCARCPDHYLTDSWHTSDECLYCSPVCKELYVKQECNRT    100

                                                                          150
muosteo.frg  HNRVCECEEGRYLELEFCLKHRSCPPPGSGVVQAGTPERNTVCKKCPDGFF  150
ratosteo.frg HNRVCECEEGRYLELEFCLKHRSCPPPGLGVLQAGTPERNTVCKRCPDGFF  150
huosteo.frg  HNRVCECKEGRYLEIEFCLKHRSCPPPGVGVVQAGTPERNTVCKRCPDGFF  150

                                                                          200
muosteo.frg  SGETSSKAPCLKHTNCSTFGLLLIQKGNATHDNVCSGNREATQKCGIDVT   200
ratosteo.frg SGETSSKAPCRKHTNCSSLGLLLQKGNATHDNVCSGNREATQNCGIDVT    200
huosteo.frg  SWETSSKAPCRKKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVT  200
```

FIG.9F

```
                                                                              250
muosteo.frg  L C E E A F F R F A V P T K I I P N W L S V L V D S L P G T K V N A E S V E R I K R R H S S Q E Q T   250
ratosteo.frg L C E E A F F R F A V P T K I I P N W L S V L V D S L P G T K V N A E S V E R I K R R H S S Q E Q T   250
huosteo.frg  L C E E A F F R F A V P T K F T P N W L S V L V D N L P G T K V N A E S V E R I K R Q H S S Q E Q T   250

                                                                              300
muosteo.frg  F Q L L K L W K H Q N R D Q E M V K K I I Q D I D L C E S S V Q R H L G H N L T T E Q L L A L H E   300
ratosteo.frg F Q L L K L W K H Q N R D Q E M V K K I I Q D I D L C E S S V Q R H I G H A N L T T E Q L R L H E   300
huosteo.frg  F Q L L K L W K H Q N K D Q D T V K K I I Q D I D L C E N S V Q R H I G H A N L T F E Q L R S L H E  300

                                                                              350
muosteo.frg  S L P G K K I S P E E I E R T R K T C K S S E Q L L K L L S L W R I K N G D Q D T L K G L H Y A L K   350
ratosteo.frg S L P G K K I S P D E I E R T R K T K A C K P S E Q L L K L L S L W R I K N G D D D T L K G L H Y A L K 350
huosteo.frg  S L P G K K V G A E D I E K T T K A C K P S D Q T L K L L S L W R I K N G D D D T L K G L H A L K   350

                                                                              400
muosteo.frg  H L K T S H F P K T V T H S L R K T H R F L H S F T H Y R L Y Q K L F L E M I G N Q V Q S V K I S C   400
ratosteo.frg H L K A V H F P K T V T H S L R K T I R F L H S F T H Y R L Y Q K L F L E M I G N Q V Q S V K I S C   400
huosteo.frg  H S K T Y H F P K I V T Q S L H K T I Q S L R K F T H Y R L Y Q K L F L E M I G N Q V Q S V K I S C 400

                                                                              401
muosteo.frg  L   401
ratosteo.frg L   401
huosteo.frg  L   401
```

FIG.10

```
                                                                                    49
                                                                                    49

ltnrr  CPQ-GKYIHPQNNSICCTKCHKGTYLYNDCPGPGQDTDCRECESGSFTAS
humoste PLPKYLHYDEETSHQLLCDKCPPGTYLPGTYLKQHCTAK-WKTVCAPCPDHYLTDS

                                                                                    98
                                                                                    93

ltnrr  ENHLRHCLSCSI-KCRKEWGQVEISSCTVDRDTVCGCRKNQYRHYWSENLF
humoste WHTSDECLYCSPVC-IKELQYVK-QECNRTHNRVCECKEGRYLEI---E-F

                                                                                    139
                                                                                    139

ltnrr  QCPNCSLCLNG-TVHLSCQEKQNTVCT-CHAGFFLRE----NECVSC
humoste -CLKHRSCPPGFGWVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKH
```

FIG.11

FIG.12A

N TERMINAL

DOMAIN 1

22-ETFPFKYLHYDEETSHQLLCDKCPPGTYLKQHCTAKWKTVCAP-64 HUMAN
22-ETLPFKYLHYDPETGHQLLCDKCAPGTYLKQHCTVRRKTLCVP-64 MOUSE

SS1   SS2   SS3

DOMAIN 2

65-CPDHYYTDSWHTSDECLYCSPVCKELQVVKQECNRTHNRVC-105
65-CPDHSYTDSWHTSDECVYCSPVCKELQSVKQECNRTHNRVC-105

SS1   SS2   SS3

DOMAIN 3

106-ECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVC-142
106-ECEEGRYLEIEFCLKHRSCPPGSGVVQAGTPERNTVC-142

SS1   SS3

DOMAIN 4

143-KRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQK-194
143-KRCPDGFFSGETSSKAPCIKHTNCSTFGLLLIQKGNATHDNVCSGNREATQK-194

SS1   SS3

FIG. 12B

```
                                                                    C
                                                                 ⎫ TERMINAL
                                                                 ⎬
                                                                 ⎭

195-[C]GIDVTI[C]ERAFFRPAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSS-246
195-[C]GIDVTI[C]ERAFFRPAVPTKIIPNWLSVLVDSLPGTKVNAESVERIKRRHSS-246

247-QEQTFQLLKLWKHQNKDQDIVKKIIQDIDL[C]ENSVQRHIGHANLTFEQLRSL-298
247-QEQTFQLLKLWKHQNRDQEMVKKIIQDIDL[C]ESSVQRHLGHSNLTEQLLAL-298

299-MESLPGKKVGAEDIEKTIKA[C]KFSDQILKLLSLWRIKNGDQDTLKGLMHALK-350
299-MESLPGKKISPEEIERTRKT[C]KSSEQLLKLLSLWRIKNGDQDTLKGLMYALK-350

351-HSKTYHPFKTVTQSLKKTIRPLHSFTMYRLYQKLFLEMIGNQVQSVKISC[L]-401
351-HLKTSHFPKTVTHSLRKTMRPLHSFTMYRLYQKLFLEMIGNQVQSVKISC[L]-401
```

FIG.13A

FIG.13B

FIG.13C

FIG.14A

Ndel cohesive end

Kpn I cohesive end

TATGGATGAAGAAACTTCTCATCAGCTGCTGTGTGATAAATGTCCGCCGGGTAC
ACCTACTTCTTTGAAGAGTAGTCGACGACACTATTTACAGGCGGCC

1257-20 hu Osteoprotegerin PCR Product

+

BamHI

#1257-19

KpnI

Oligo Linker & PCR Product

pAMG21

Ndel BamHI

A fragment

BamHI

KpnI

Ndel

pAMG21-human -
OSTEOPROTEGERIN -
32-401

FIG.14B

FIG.15

110 kd

55 kd
50 kd

FIG.16A

Cell Lysate   Medium

Non-Reducing

-100 K ─   dimer

-55 K ═   monomer

FIG.16B

Reducing

-55 K ═   monomer

FIG.17

CTLL-2 Media

CHO mu OPG [22-401] Media

◄ Dimer

◄ Monomer

FIG.18

FIG.19B

OPG - 10 ng/ml

FIG.19D

OPG - 0.1 ng/ml

FIG.19A

OPG - 100 ng/ml

FIG.19C

OPG - 1 ng/ml

FIG.19F

OPG - 0.001 ng/ml

FIG.19E

OPG - 0.01 ng/ml

FIG.19G

Control
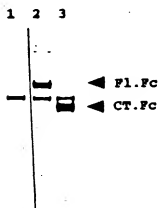
FIG.20

FIG.21

Legend

Growth	Intermediate	Terminal
Bone marrow cells CSF -1	PGE2 + CSF-1	ST2 cells 1,25 (OH)2 D3 Dexamethasone
4 days	2 days	8 - 10 days

Groups	OPG	OPG
CTL - CTL	– – –	– – –
OPG - CTL	100 ng/ml	– – –
OPG - OPG	– – –	100 ng/ml
OPG - OPG	100 ng/ml	100 ng/ml

FIG.22A
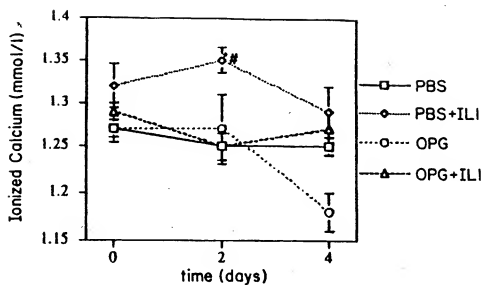
FIG.22B
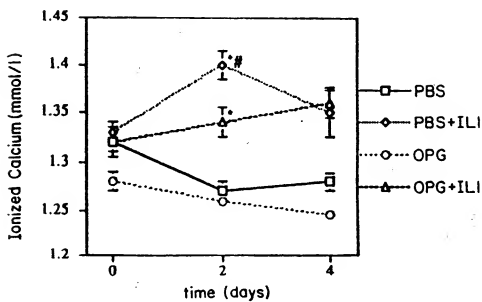
* Different to PBS, p < 0.05
# Different to OPG + IL1, p < 0.05

FIG.23A

PBS/PBS
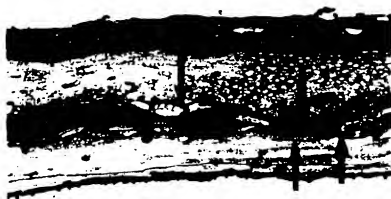
FIG.23B

FIG.23C

PBS/OPG

FIG.23D
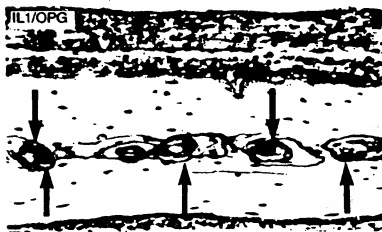
FIG.24B

FIG.24A
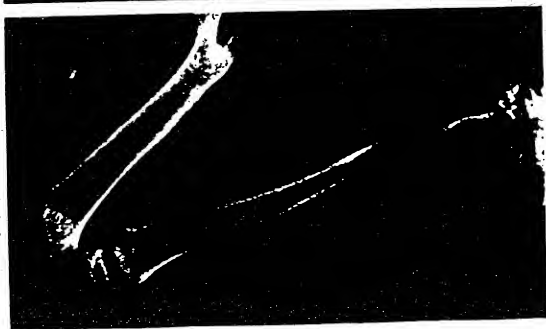
FIG.25
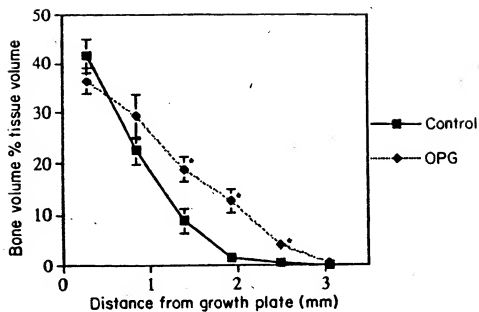
* Different to control p < 0.01

FIG.26A   FIG.26.B

FIG.27
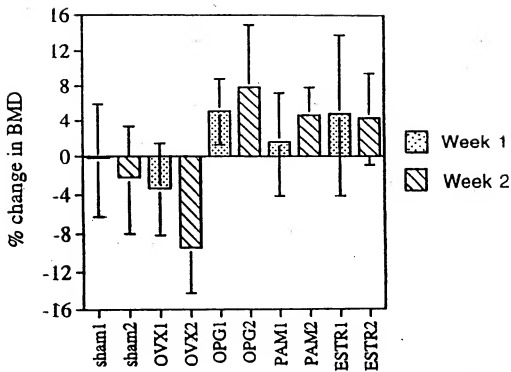
FIG.28
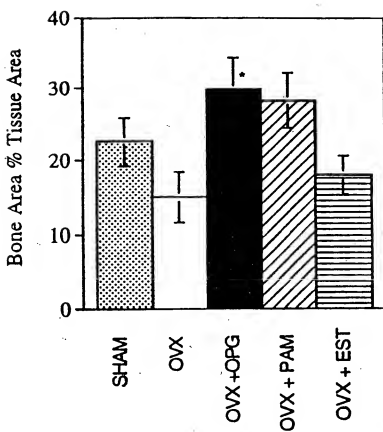
* Different to OVX p < 0.05

Figure 29A

```
              DraIII
                |
CATGGGAAATGTCAGAGTGGAGAACCACACCGAGTGCCACTGCAGCACTTGTTATTATCA
1 ----------+---------+---------+---------+---------+---------+ 60
GTACCCTTTACAGTCTCACCTCTTGGTGTGGCTCACGGTGACGTCGTGAACAATAATAGT

CAAATCCTAATAGTTTGCAGTGGGCCTTGCTGATGATGGCTGACTTGCTCAAAAGGAAAA
61 ----------+---------+---------+---------+---------+---------+ 120
GTTTAGGATTATCAAACGTCACCCGGAACGACTACTACCGACTGAACGAGTTTTCCTTTT

TTAATTTGTCCAGTGTCTATGGCTTTGTGAGATAAAACCCTCCTTTTCCTTGCCATACCA
121 ----------+---------+---------+---------+---------+---------+ 180
AATTAAACAGGTCACAGATACCGAAACACTCTATTTTGGGAGGAAAAGGAACGGTATGGT

TTTTTAACCTGCTTTGAGAATATACTGCAGCTTTATTGCTTTTCTCCTTATCCTACAATA
181 ----------+---------+---------+---------+---------+---------+ 240
AAAAATTGGACGAAACTCTTATATGACGTCGAAATAACGAAAAGAGGAATAGGATGTTAT

TAATCAGTAGTCTTGATCTTTTCATTTGGAATGAAATATGGCATTTAGCATGACCATAAA
241 ----------+---------+---------+---------+---------+---------+ 300
ATTAGTCATCAGAACTAGAAAAGTAAACCTTACTTTATACCGTAAATCGTACTGGTATTT

AAGCTGATTCCACTGGAAATAAAGTCTTTTAAATCATCACTCTATCACTGAATTCTAATT
301 ----------+---------+---------+---------+---------+---------+ 360
TTCGACTAAGGTGACCTTTATTTCAGAAAATTTAGTAGTGAGATAGTGACTTAAGATTAA

TTTTCTGAAAAGTTTCAAGCCAGTTACTTTTGATAGGATTAACGGAAGGGAGTGAGCCAG
361 ----------+---------+---------+---------+---------+---------+ 420
AAAAGACTTTTCAAAGTTCGGTCAATGAAAACTATCCTAATTGCCTTCCCTCACTCGGTC

               XcmI
                |
TGGGTGAGGTGGGTTCCCATGTAGTCAATGGCCTAATACTGGAGAATCTTATTCTAACCA
421 ----------+---------+---------+---------+---------+---------+ 480
ACCCACTCCACCCAAGGGTACATCAGTTACCGGATTATGACCTCTTAGAATAAGATTGGT

AGCCTTCCAGAGCAAGCTGTGAGCCCCTCAGACAGTGGGCTACTCATGAGACAGTCCATT
481 ----------+---------+---------+---------+---------+---------+ 540
TCGGAAGGTCTCGTTCGACACTCGGGGAGTCTGTCACCCGATGAGTACTCTGTCAGGTAA

GGGGTAAAGGAAGAAAATATAACTTCTATTTCTATTCATTTGCACATTGTCTTTAGATGC
541 ----------+---------+---------+---------+---------+---------+ 600
CCCCATTTCCTCTTCTTTATATTGAAGATAAAGATAAGTAAACGTGTAACAGAAATCTACG

CCATTTGGGTGAGTTTTATAGAAGTACAGCTACATTAAAAAATAGAACTGATAATAGATA
601 ----------+---------+---------+---------+---------+---------+ 660
GGTAAACCACTCAAAATATCTTCATGTCGATGTAATTTTTATCTTGACTATTATCTAT

AGGCTTTAAAAAAACTTCATTCATCACCAGTTTGTCAAGATTCCATTTCAAAGTGAAAAA
661 ----------+---------+---------+---------+---------+---------+ 720
TCCGAAATTTTTTGAAGTAAGTAGTGGTCAAACAGTTCTAAGGTAAAGTTTCACTTTTT

CCAATTTCTAACGGGTTGGTAAACACAGCAGATGGCAGGGTGAAAAATTAAAGTGAGTGC
721 ----------+---------+---------+---------+---------+---------+ 780
GGTTAAAGATTGCCCAACCATTTGTGTCGTCTACCGTCCCACTTTTTAATTTCACTCACG

ATGTACCTTTAAAGAAACACTGAAATGCACACACATTACTTAACCTGCTCATTCATTTAT
781 ----------+---------+---------+---------+---------+---------+ 840
TACATGGAAATTTCTTTGTGACTTTACGTGTGTGTAATGAATTGGACGAGTAAGTAAATA

TTACATATAGTCTTGGGTGTACAAAATTTAGAAATAAATACATATGGGGGCGGGGCCTTA
841 ----------+---------+---------+---------+---------+---------+ 900
AATGTATATCAGAACCCACATGTTTTAAATCTTTATTTATGTATACCCCGCCCCGGAAT

GCTGCACAAATAGGATGCGCGGGCGGGCCTTGGTAGGGGCGGAGCCTTAGCTGCACAAATA
901 ----------+---------+---------+---------+---------+---------+ 960
CGACGTGTTTATCCTACGCGCCGCCCGGAACCATCCCCGCCTCGGAATCGACGTGTTTAT

GGATGCGCGGCGGGCCTTGGTGGGGGCGGGGCCTAAGCTGCGCAAGTGGTACACAGCTCA
961 ----------+---------+---------+---------+---------+---------+ 1020
CCTACGCGCCGCCCGGAACCACCCCCGCCCCGGATTCGACGCGTTCACCATGTGTCCGAGT

GGGCTGCGATTTCGCGCCAAACTTGACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTA
1021 ----------+---------+---------+---------+---------+---------+ 1080
CCCGACGCTAAAGCGCGGTTTGAACTGCCGTTAGGATCGCCACTTCCGACCATCCTAAAAT
```

Figure 29B

```
    TCCCCGCTGCCATCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGG
1081 ·····----·----+----·----+----·----+----·----+----·----+ 1140
    AGGGGCGACGGTAGTACCAAGCTGGTAACTTGACGTAGCAGCGGCACAGGGTTTTATACC

    GGATTGGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAAGGAGTTCAAGTACTTCC
1141 ·····----·----+----·----+----·----+----·----+----·----+ 1200
    CCTAACCGTTCTTGCCTCTGGATGGGACCGGAGGCGAGTCCTTGCTCAAGTTCATGAAGG

    AAAGAATGACCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGA
1201 ·····----·----+----·----+----·----+----·----+----·----+ 1260
    TTTCTTACTGGTGTTGGAGAAGTCACCTTCCATTTGTCTTAGACCACTAATACCCATCCT

    AAACCTGGTTCTCCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTC
1261 ·····----·----+----·----+----·----+----·----+----·----+ 1320
    TTTGGACCAAGAGGTAAGGACTCTTCTTAGCTGGAAATTTCCTGTCTTAATTATATCAAG

              SacI        BstXI
                |           |
    TCAGTAGAGAACTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATG
1321 ·····----·----+----·----+----·----+----·----+----·----+ 1380
    AGTCATCTCTTGAGTTTCTTGGTGGTGCTCCTCGAGTAAAAGAACGGTTTCAAACCTAC

    AflIII
      |
    ATGCCTTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAG
1381 ·····----·----+----·----+----·----+----·----+----·----+ 1440
    TACGGAATTCTGAATAACTTGTTGGCCTTAACCGTTCATTTCATCTGTACCAAACCTATC

    TCGGAGGCAGTTCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTCAGACTCTTTG
1441 ·····----·----+----·----+----·----+----·----+----·----+ 1500
    AGCCTCCGTCAAGACAAATGGTCCTTCGGTACTTAGTTGGTCCGGTGGAGTCTGAGAAAC

    TGACAAGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGA
1501 ·····----·----+----·----+----·----+----·----+----·----+ 1560
    ACTGTTCCTAGTACGTCCTTAAACTTTCACTGTGCAAAAAGGGTCTTTAACTAAACCCCT

    AATATAAACTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCA
1561 ·····----·----+----·----+----·----+----·----+----·----+ 1620
    TTATATTTGAAGAGGGTCTTATGGGTCCGCAGGAGAGACTCCAGGTCCTCCTTTTTCCGT

    TCAAGTATAAGTTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCT
1621 ·····----·----+----·----+----·----+----·----+----·----+ 1680
    AGTTCATATTCAAACTTCAGATGCTCTTCTTTCTGATTGTCCTTCTACGAAAGTTCAAGA

                          BglII
                            |
    CTGCTCCCCTCCTAAAGCTATGCATTTTTATAAGACCATGGGACTTTTGCTGGCTTTAGA
1681 ·····----·----+----·----+----·----+----·----+----·----+ 1740
    GACGAGGGGACGATTTCGATACGTAAAAATATTCTGGTACCCTGAAAACGACCGGAAATCT

    TCTGAAACACTGAAATTGTCTGCTTCTCATCTTCAGTGAGATTCCAAAGGATAGTACAGT
1741 ·····----·----+----·----+----·----+----·----+----·----+ 1800
    AGACTTTGTGACTTTAACAGACGAAGAGTAGAAGTCACTCTAAGGTTTCCTATCATGTCA

    GACAGAACAAGAATAGGCACTCTCTACAAAAAAAAGAAAGAAAAAACTAAGTAATAGCAA
1801 ·····----·----+----·----+----·----+----·----+----·----+ 1860
    CTGTCTTGTTCTTATCCGTGAAGAGATGTTTTTTTCTTTCTTTTTTGATTCATTATCGTT

    GCATAATAGCTACTGTTAAGAACTCAGAGATAATGAATTGAGAATGGATACTGCTTGAAA
1861 ·····----·----+----·----+----·----+----·----+----·----+ 1920
    CGTATTATCGATGACAATTCTTGAGTCTCTATTACTTAACTCTTACCTATGACGAACTTT

    TGAAAATTTAATAAGTTAGAAACTAAACTTTATAAAAATAAAAAAATGAGCATTAAAAAA
1921 ·····----·----+----·----+----·----+----·----+----·----+ 1980
    ACTTTTAAATTATTCAATCTTTGATTTGAAATATTTTTATTTTTTTTACTCGTAATTTTTT

                        NheI
                          |
    AAAAAAAAAAAAAAAAAAAACCCCCCCCCCCCCCCCCTGCAGCCAAGCTAGCTTGGAATC
1981 ·····----·----+----·----+----·----+----·----+----·----+ 2040
    TTTTTTTTTTTTTTTTTTTTGGGGGGGGGGGGGGGGGACGTCGGTTCGATCGAACCTTAG

        BspLU11I
            |
    AGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAA
```

Figure 29C

```
2041 --------+---------+---------+---------+---------+---------+ 2100
     TCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCGTTTTCCGGTCCTTGGCATT

     AAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAA
2101 --------+---------+---------+---------+---------+---------+ 2160
     TTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTT

     TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCC
2161 --------+---------+---------+---------+---------+---------+ 2220
     AGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGG

     CCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC
2221 --------+---------+---------+---------+---------+---------+ 2280
     GGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAG

     CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAG
2281 --------+---------+---------+---------+---------+---------+ 2340
     GCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTGCGACATCCATAGAGTC

     TTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA
2341 --------+---------+---------+---------+---------+---------+ 2400
     AAGCCACATCCAGCAAGCGAGGTTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCT

     CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATC
2401 --------+---------+---------+---------+---------+---------+ 2460
     GGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGGCCATTCTGTGCTGAATAG

     GCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTAC
2461 --------+---------+---------+---------+---------+---------+ 2520
     CGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCTCCATACATCCGCCACGATG

     AGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTG
2521 --------+---------+---------+---------+---------+---------+ 2580
     TCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCCTGTCATAAACCATAGAC

     CGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACA
2581 --------+---------+---------+---------+---------+---------+ 2640
     GCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCATCGAGAACTAGGCCGTTTGT

     HgiEII
       |
     AACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAA
2641 --------+---------+---------+---------+---------+---------+ 2700
     TTGGTGGCGACCATCGCCACCAAAAAAACAAACGTTCGTCGTCTAATGCGCGTCTTTTTT

     AGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAA
2701 --------+---------+---------+---------+---------+---------+ 2760
     TCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTT

     CTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
2761 --------+---------+---------+---------+---------+---------+ 2820
     GAGTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCTAGAAGTGGATCTAGGAAAA

     AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAG
2821 --------+---------+---------+---------+---------+---------+ 2880
     TTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATATACTCATTTGAACCAGACTGTC

     TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
2881 --------+---------+---------+---------+---------+---------+ 2940
     AATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTA

     AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCC
2941 --------+---------+---------+---------+---------+---------+ 3000
     TCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCCCTCCCGAATGGTAGACCGGG

     CAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAA
3001 --------+---------+---------+---------+---------+---------+ 3060
     GTCACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAGGTCTAAATAGTCGTTATTT

     CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCA
3061 --------+---------+---------+---------+---------+---------+ 3120
     GGTCGGTCGGCCTTCCCGGCTCGCGTCTTCACCAGGACGTTGAAATAGGCGGAGGTAGGT

     GTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAA
3121 --------+---------+---------+---------+---------+---------+ 3180
     CAGATAATTAACAACGGCCCTTCGATCTCATTCATCAAGCGGTCAATTATCAAACGCGTT
```

Figure 29D

```
   CGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT
3181 ----------+---------+---------+---------+---------+---------+ 3240
   GCAACAACGGTAACGACGTCCGTAGCACCACAGTGCGAGCAGCAAACCATACCGAAGTAA

   CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGC
3241 ----------+---------+---------+---------+---------+---------+ 3300
   GTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGGGGTACAACACGTTTTTTCG

                  EaeI
           PvuI   GdiII
             |      |
   GGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACT
3301 ----------+---------+---------+---------+---------+---------+ 3360
   CCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCAACCGGCGTCACAATAGTGA

   CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTC
3361 ----------+---------+---------+---------+---------+---------+ 3420
   GTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAG

                  BcqI
                    |
   TGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTG
3421 ----------+---------+---------+---------+---------+---------+ 3480
   ACACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCACATACGCCGCTGGCTCAAC

   CTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCT
3481 ----------+---------+---------+---------+---------+---------+ 3540
   GAGAACGGGCCGCAGTTGTGCCCTATTATGGCGCGGTGTATCGTCTTTGAAATTTTCACGA

   CATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATC
3541 ----------+---------+---------+---------+---------+---------+ 3600
   GTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCTAGAATGGCGACAACTCTAG

   CAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
3601 ----------+---------+---------+---------+---------+---------+ 3660
   GTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTCGTAGAAAATGAAAGTGGTC

   CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGAC
3661 ----------+---------+---------+---------+---------+---------+ 3720
   GCAAAGACCCACTCGTTTTTGTCCTTCCGTTTTACGGCGTTTTTTCCCTTATTCCCGCTG

           SspI
             |
   ACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGG
3721 ----------+---------+---------+---------+---------+---------+ 3780
   TGCCTTTACAACTTATGAGTATGAGAAGGAAAAAGTTTATAATAACTTCGTAAATAGTCCC

   TTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGT
3781 ----------+---------+---------+---------+---------+---------+ 3840
   AATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCCA

   TCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGAC
3841 ----------+---------+---------+---------+---------+---------+ 3900
   AGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGATTCTTTGGTAATAATAGTACTG

   ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCTTCAAGAATTCCCTGTGGA
3901 ----------+---------+---------+---------+---------+---------+ 3960
   TAATTGGATATTTATCTTATCCGCATAGTGCTCCGGGAAAGCAGAAGTTCTTAAGGGACACCT

   ATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAA
3961 ----------+---------+---------+---------+---------+---------+ 4020
   TACACACAGTCAATCCCACACCTTTCAGGGGTCCGAAGGGGTGTCCGTCTTCATACGTTT

   GCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCA
4021 ----------+---------+---------+---------+---------+---------+ 4080
   CGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGT

   GAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGC
4081 ----------+---------+---------+---------+---------+---------+ 4140
   CTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCG

   CCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTT
4141 ----------+---------+---------+---------+---------+---------+ 4200
   GGTAGGGCGGGGATTGAGGCGGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAA
```

Figure 29E

```
              SfiI
              |
  \ TTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAG
4201 ------------------------------------------------------------+ 4260
    AAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTC

          AvrII
          |
  GAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTGGTCGAGGCTCGCATCTCTCCTT
4261 ------------------------------------------------------------+ 4320
    CTCCGAAAAAACCTCCGGATCCGAAAACGTTTTTCGACCAGCTCCGAGCGTAGAGAGGAA

  CACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGC
4321 ------------------------------------------------------------+ 4380
    GTGCGCGGGCGGCGGGATGGACTCCGGCGGTAGGTGCGGCCAACTCAGCGCAAGACGGCG

  CTCCCGCCTGTGGTGGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTTAAAGCTCAGGT
4381 ------------------------------------------------------------+ 4440
    GAGGGCGGACACCACGGAGGACTTGACGCAGGCGGCAGATCCATTCAAATTTCGAGTCCA

                    NgoAIV
                    |
  CGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCA
4441 ------------------------------------------------------------+ 4500
    GCTCTGGCCCGGAAACAGGCCGCGAGGGAACCTCGGATGGATCTGAGTCGGCCGAGAGGT

  CGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCC
4501 ------------------------------------------------------------+ 4560
    GCGAAACGGACTGGGACGAACGAGTTGAGATGCAGAAACAAAGCAAAAGACAAGACGCGG

                HpaI
                |
  GTTACAGATCCGTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTTTT
4561 ------------------------------------------------------------+ 4620
    CAATGTCTAGGCAGCTCCTTGACTTTTTGGTCTTTCAATTGACCATTCAAATCAGAAAAA

       Psp5II BamHI
       |    |
  GTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTG
4621 ------------------------------------------------------------+ 4680
    CAGAAAATAAAGTCCAGGGCCTAGGCCACCACCACGTTTAGTTCTTGACGAGGAGTCAC

  GATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCTGC
4681 ------------------------------------------------------------+ 4740
    CTACAACGGAAATGAAGATCCGGACATGCCTTCACAATGAAGACGAGATTTTCGACGACG

  HindIII XbaI           BssHII
  |       |              |  |
  AACAAGCTTCTAGACCACCATGAACAAGTTGCTGTGCTGCGCGCTCGTGTTTCTGGACAT
4741 ------------------------------------------------------------+ 4800
    TTGTTCGAAGATCTGGTGGTACTTGTTCAACGACACGACGCGCGAGCACAAAGACCTGTA
 b            M  N  K  L  L  C  C  A  L  V  F  L  D  I  -

  CTCCATTAAGTGGACCACCCAGGAAACGTTTCCTCCAAAGTACCTTCATTATGACGAAGA
4801 ------------------------------------------------------------+ 4860
    GAGGTAATTCACCTGGTGGGTCCTTTGCAAAGGAGGTTTCATGGAAGTAATACTGCTTCT
 b     S  I  K  W  T  T  Q  E  T  F  P  P  K  Y  L  H  Y  D  E  E  -

                 KpnI
                 |
  AACCTCTCATCAGCTGTTGTGTGACAAATGTCCTCCTGGTACCTACCTAAAACAACACTG
4861 ------------------------------------------------------------+ 4920
    TTGGAGAGTAGTCGACAACACACTGTTTACAGGAGGACCATGGATGGATTTTGTTGTGAC
 b   T  S  H  Q  L  L  C  D  K  C  P  P  G  T  Y  L  K  Q  H  C  -

  TACAGCAAAGTGGAAGACCGTGTGCGCCCCTTGCCCTGACCACTACTACACAGACAGCTG
4921 ------------------------------------------------------------+ 4980
    ATGTCGTTTCACCTTCTGGCACACGCGGGGAACGGGACTGGTGATGATGTGTCTGTCGAC
 b   T  A  K  W  K  T  V  C  A  P  C  P  D  H  Y  Y  T  D  S  W  -

  GCACACCAGTGACGAGTGTCTATACTGCAGCCCCGTGTGCAAGGAGCTGCAGTACGTCAA
4981 ------------------------------------------------------------+ 5040
    CGTGTGGTCACTGCTCACTGATATGACGTCGGGGCACACGTTCCTCGACGTCATGCAGTT
 b   H  T  S  D  E  C  L  Y  C  S  P  V  C  K  E  L  Q  Y  V  K  -

              RleAI BamI
```

Figure 29F

```
                        |  |
      GCAGGAGTGCAATCGCACCCACAACCGCGTGTGCGAATGCAAGGAAGGGCGCTACCTTGA
 5041 ----------+---------+---------+---------+---------+---------+ 5100
      CGTCCTCACGTTAGCGTGGGTGTTGGCGCACACGCTTACGTTCCTTCCCCGCGATGGAACT
 b    Q E C N R T H N R V C E C K E G R Y L E ·

      GATAGAGTTCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTTGGAGTGGTGCAAGCTGG
 5101 ----------+---------+---------+---------+---------+---------+ 5160
      CTATCTCAAGACGAACTTTGTATCCTCGACGGGAGGACCTAAACCTCACCACGTTCGACC
 b    I E F C L K H R S C P P G F G V V Q A G ·

                            BsmBI
                              |
      AACCCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTTCTTCTCAAATGAGAC
 5161 ----------+---------+---------+---------+---------+---------+ 5220
      TTGGGGTCTCGCTTTATGTCAAACGTTTTCTACAGGTCTACCCAAGAAGAGTTTACTCTG
 b    T P E R N T V C K R C P D G F F S N E T ·

      GTCATCTAAAGCACCCTGTAGAAAACACACAAATTGCAGTGTCTTTGGTCTCCTGCTAAC
 5221 ----------+---------+---------+---------+---------+---------+ 5280
      CAGTAGATTTCGTGGGACATCTTTTGTGTGTTTAACGTCACAGAAACCAGAGGACGATTG
 b    S S K A P C R K H T N C S V F G L L L T ·

                        BspEI
                          |
      TCAGAAAGGAAATGCAACACACGACAACATATGTTCCGGAAACAGTGAATCAACTCAAAA
 5281 ----------+---------+---------+---------+---------+---------+ 5340
      AGTCTTTCCTTTACGTTGTGTGCTGTTGTATACAAGGCCTTTGTCACTTAGTTGAGTTTT
 b    Q K G N A T H D N I C S G N S E S T Q K ·

      SalI          BmgI
       |              |
      AGTCGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTC
 5341 ----------+---------+---------+---------+---------+---------+ 5400
      TCAGCTGTTTTGAGTGTGTACGGGTGGCACGGGTCGTGGACTTGAGGACCCCCCTGGCAG.
 b    V D K T H T C P P C P A P E L L G G P S ·

      AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGT
 5401 ----------+---------+---------+---------+---------+---------+ 5460
      TCAGAAGGAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCA
 b    V F L F P P K P K D T L M I S R T P E V ·

          BtrI
           |
      CACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
 5461 ----------+---------+---------+---------+---------+---------+ 5520
      GTGTACGACACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCA
 b    T C V V V D V S H E D P E V K F N W Y V ·

                    SacII
                     |
      GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAC
 5521 ----------+---------+---------+---------+---------+---------+ 5580
      CCTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTG
 b    D G V E V H N A K T K P R E E Q Y N S T ·

      GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTA
 5581 ----------+---------+---------+---------+---------+---------+ 5640
      CATGGCACACCAGTCGCAGGAGTGGCAGGAGCGTGGTCCTGACCGTTACCGTTCCTCAT
 b    Y R V V S V L T V L H Q D W L N G K E Y ·

      CAAGTGCAAAGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGC
 5641 ----------+---------+---------+---------+---------+---------+ 5700
      GTTCACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCG
 b    K C K V S N K A L P A P I E K T I S K A ·

                          SmaI
                            |
      CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGAC
 5701 ----------+---------+---------+---------+---------+---------+ 5760
      GTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGACGGGGGTAGGGCCCTACTCGACTG
 b    K G Q P R E P Q V Y T L P P S R D E L T ·

      CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGT
 5761 ----------+---------+---------+---------+---------+---------+ 5820
      GTTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCA
 b    K G Q P R E P Q V Y T L P P S R D E L T ·
```

Figure 29G

```
b      K N Q V S L T C L V K G F Y P S D I A V ·

    GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGA
5831 ----------+---------+---------+---------+---------+---------+ 5880
    CCTCACCCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGCGGAGGGCACGACCT
b      E W E S N G Q P E N N Y K T T P P V L D ·

                           AarI
                            |
    CTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA
5881 ----------+---------+---------+---------+---------+---------+ 5940
    GAGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACCTGTTCTCGTCCACCGTCGT
b'     S D G S F F L Y S K L T V D K S R W Q Q ·

                           SapI
                            |
    GGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
5941 ----------+---------+---------+---------+---------+---------+ 6000
    CCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTT
b      C N V F S C S V M H E A L H N H Y T Q K ·

    GAGCCTCTCCCTGTCTCCGGGTAAATGATAACTCGAC
6001 ----------+---------+---------+------- 6037
    CTCGGAGAGGGACAGAGGCCCATTTACTATTGAGCTG
b      S L S L S P G K * *
```

Figure 30A

Effects of OPG-Fc during the course of adjuvant arthritis in male Lewis rats (Expt. AdA-14, D9-D16)
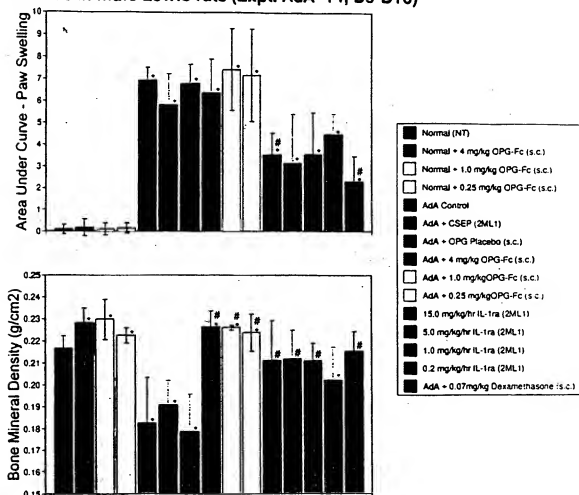
Legend:
- Normal (NT)
- Normal + 4 mg/kg OPG-Fc (s.c.)
- Normal + 1.0 mg/kg OPG-Fc (s.c.)
- Normal + 0.25 mg/kg OPG-Fc (s.c.)
- AdA Control
- AdA + CSEP (2ML1)
- AdA + OPG Placebo (s.c.)
- AdA + 4 mg/kg OPG-Fc (s.c.)
- AdA + 1.0 mg/kgOPG-Fc (s.c.)
- AdA + 0.25 mg/kgOPG-Fc (s.c.)
- 15.0 mg/kg/hr IL-1ra (2ML1)
- 5.0 mg/kg/hr IL-1ra (2ML1)
- 1.0 mg/kg/hr IL-1ra (2ML1)
- 0.2 mg/kg/hr IL-1ra (2ML1)
- AdA + 0.07mg/kg Dexamethasone (s.c.)

Paws from rats with adjuvant arthritis induced by 0.5mg mycobacteria in oil were analyzed by DEXA for BMD. Evaluation of BMD, a 29mm X 25mm box was centered at the calcaneus (expt AdA-14 2/99, Amgen nb#22957 p47-49). * compared to normal, # compared to vehicle
P < 0.05 Mann-Whitney U test

Figure 30B

Effects of OPG-Fc during the course of adjuvant arthritis in male Lewis rats (Expt. AdA- 17, D9-D16)
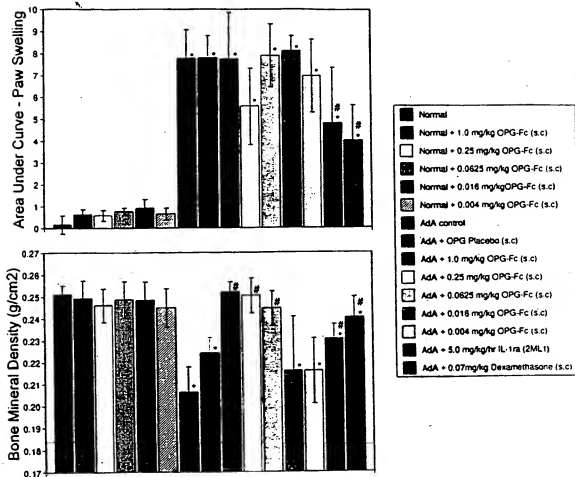
Legend:
- Normal
- Normal + 1.0 mg/kg OPG-Fc (s.c)
- Normal + 0.25 mg/kg OPG-Fc (s.c)
- Normal + 0.0625 mg/kg OPG-Fc (s.c)
- Normal + 0.016 mg/kgOPG-Fc (s.c)
- Normal + 0.004 mg/kg OPG-Fc (s.c)
- AdA control
- AdA + OPG Placebo (s.c)
- AdA + 1.0 mg/kg OPG-Fc (s.c)
- AdA + 0.25 mg/kg OPG-Fc (s.c)
- AdA + 0.0625 mg/kg OPG-Fc (s.c)
- AdA + 0.016 mg/kg OPG-Fc (s.c)
- AdA + 0.004 mg/kg OPG-Fc (s.c)
- AdA + 5.0 mg/kg/hr IL-1ra (2ML1)
- AdA + 0.07mg/kg Dexamethasone (s.c)

Paws from rats with adjuvant arthritis induced by 0.5mg mycobacteria in oil were analyzed by DEXA for BMD. Evaluation of BMD, a 29mm X 25mm box was centered at the calcaneus (expt AdA -17 3/99, Amgen nb#22957 p62-65).
* compared to normal, # compared to vehicle
P < 0.05 Mann-Whitney U test

Figure 31A

Combination treatment with OPG-Fc and IL-1ra or s-TNF-R1 on adjuvant arthritis in male Lewis rats (AdA-20, D9-D15)

■ NT	■ 1.0 mg/kg s-TNF-R1
■ AdA	■ 0.03 mg/kg OPG-Fc
■ Vehicle	▨ 0.2 mg/kg/hr IL-1ra + 0.03 mg/kg OPG-Fc
■ 0.2 mg/kg/hr IL-1ra	☐ 1.0 mg/kg/hr IL-1ra + 0.03 mg/kg OPG-Fc
■ 1.0 mg/kg/hr IL-1ra	☐ 0.25 mg/kg s-TNF-R1 + 0.03 mg/kg OPG-Fc
■ 0.25 mg/kg s-TNF-R1	☐ 1.0 mg/kg s-TNF-R1+ 0.03 mg/kg OPG-Fc
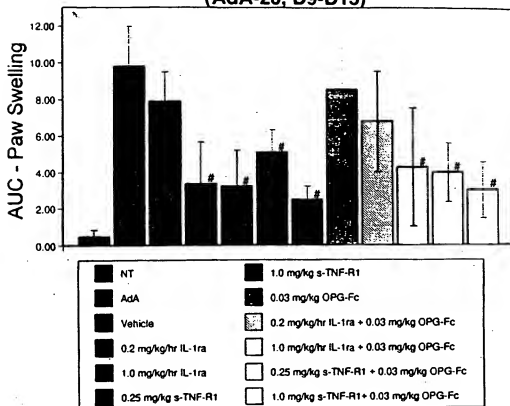
Paws from rats with adjuvant arthritis induced by 0.5mg mycobacteria in oil were
analyzed by DEXA for BMD. (expt AdA-20 5/99, Amgen nb#22957 p84).
* compared to normal, # compared to vehicle
P < 0.05 Mann-Whitney U test.
All groups are significant vs normal

Figure 31B

Combination treatment with OPG-Fc and IL-1ra or s-TNF-R1 on adjuvant arthritis in male Lewis rats (AdA-20, D9-D15)

■ NT	■ 1.0 mg/kg s-TNF-R1
■ AdA	■ 0.03 mg/kg OPG-Fc
■ Vehicle	▦ 0.2 mg/kg/hr IL-1ra + 0.03 mg/kg OPG-Fc
■ 0.2 mg/kg/hr IL-1ra	▦ 1.0 mg/kg/hr IL-1ra + 0.03 mg/kg OPG-Fc
■ 1.0 mg/kg/hr IL-1ra	□ 0.25 mg/kg s-TNF-R1 + 0.03 mg/kg OPG-Fc
■ 0.25 mg/kg s-TNF-R1	□ 1.0 mg/kg s-TNF-R1+ 0.03 mg/kg OPG-Fc
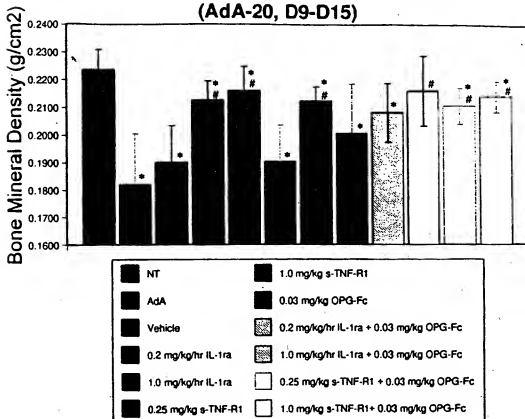
Paws from rats with adjuvant arthritis induced by 0.5mg mycobacteria in oil were analyzed by DEXA for BMD. Evaluation of BMD, a 29mm X 25mm was centered at the tibiotarsal region. (expt AdA-20 5/99, Amgen nb#22957 p88).
* compared to normal, # compared to vehicle
.P < 0.05 Mann-Whitney U test.

1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

  (i) APPLICANT:Amgen Inc.

  (ii) TITLE OF INVENTION: OSTEOPROTEGERIN

  (iii) NUMBER OF SEQUENCES: 168

  (iv) CORRESPONDENCE ADDRESS:
     (A) ADDRESSEE: Amgen Inc.
     (B) STREET: 1840 Dehavilland Drive
     (C) CITY: Thousand Oaks
     (D) STATE: California
     (E) COUNTRY: United States
     (F) ZIP: 91320

  (v) COMPUTER READABLE FORM:
     (A) MEDIUM TYPE: Floppy disk
     (B) COMPUTER: IBM PC compatible
     (C) OPERATING SYSTEM: PC-DOS/MS-DOS
     (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

  (vi) CURRENT APPLICATION DATA:
     (A) APPLICATION NUMBER:
     (B) FILING DATE:
     (C) CLASSIFICATION:

  (viii) ATTORNEY/AGENT INFORMATION:
     (A) NAME: Winter, Robert B.
     (C) REFERENCE/DOCKET NUMBER: A-378-CIP2

(2) INFORMATION FOR SEQ ID NO:1:

  (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 36 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAAGGAAGGA AAAAAGCGGC CGCTACANNN NNNNNT   36

(2) INFORMATION FOR SEQ ID NO:2:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 16 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCGACCCACG CGTCCG   16

(2) INFORMATION FOR SEQ ID NO:3:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 12 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTGCGCAG GC   12

(2) INFORMATION FOR SEQ ID NO:4:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 18 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGTAAAACGA CGGCCAGT   18

3

(2) INFORMATION FOR SEQ ID NO:5:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 18 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGGAAACAG CTATGACC   18

(2) INFORMATION FOR SEQ ID NO:6:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 20 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAATTAACCC TCACTAAAGG   20

(2) INFORMATION FOR SEQ ID NO:7:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 23 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCATTATGAC CCAGAAACCG GAC   23

(2) INFORMATION FOR SEQ ID NO:8:

  (i) SEQUENCE CHARACTERISTICS:

4

      (A) LENGTH: 23 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGGTAGCGCC CTTCCTCACA TTC   23

(2) INFORMATION FOR SEQ ID NO:9:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 30 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACTAGTCCC ACAATGAACA AGTGGCTGTG   30

(2) INFORMATION FOR SEQ ID NO:10:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 45 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAAGAATGC GGCCGCTAAA CTATGAAACA GCCCAGTGAC CATTC   45

(2) INFORMATION FOR SEQ ID NO:11:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 21 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTCTAGAA AGAGCTGGGA C   21

(2) INFORMATION FOR SEQ ID NO:12:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 21 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCCGTGTTC CATTTATGAG C   21

(2) INFORMATION FOR SEQ ID NO:13:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 24 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATCAAAGGCA GGGCATACTT CCTG   24

(2) INFORMATION FOR SEQ ID NO:14:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 24 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTTGCACTCC TGTTTCACGG TCTG   24

(2) INFORMATION FOR SEQ ID NO:15:

6

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
      ˎ (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAAGACACCT TGAAGGGCCT GATG   24

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAACTTTTAC AGAAGAGCAT CAGC   24

  (2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCGCGGCCG CATGAACAAG TGGCTGTGCT GCG   33

(2) INFORMATION FOR SEQ ID NO:18:

   (i) SEQUENCE CHARACTERISTICS:
     ˙ (A) LENGTH: 31 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

7

        (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

    AGCTCTAGAG AAACAGCCCA GTGACCATTC C   31

    (2) INFORMATION FOR SEQ ID NO:19:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

    GTGAAGCTGT GCAAGAACCT GATG   24

    (2) INFORMATION FOR SEQ ID NO:20:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

    ATCAAAGGCA GGGCATACTT CCTG   24

    (2) INFORMATION FOR SEQ ID NO:21:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

8

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAGATCCTGA AGCTGCTCAG TTTG   24

(2) INFORMATION FOR SEQ ID NO:22:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 33 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCGCGGCCG CGGGGACCAC AATGAACAAG TTG   33

  (2) INFORMATION FOR SEQ ID NO:23:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 33 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCTCTAGAA TTGTGAGGAA ACAGCTCAAT GGC   33

(2) INFORMATION FOR SEQ ID NO:24:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 39 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

9

ATAGCGGCCG CTGAGCCCAA ATCTTGTGAC AAAACTCAC   39

(2) INFORMATION FOR SEQ ID NO:25:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 45 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCTAGAGTCG ACTTATCATT TACCCGGAGA CAGGGAGAGG CTCTT   45

   (2) INFORMATION FOR SEQ ID NO:26:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 38 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

(2) INFORMATION FOR SEQ ID NO:27:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 43 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CCTCTGCGGC CGCTAAGCAG CTTATTTTCA CGGATTGAAC CTG   43

(2) INFORMATION FOR SEQ ID NO:28:

10

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 38 base pairs
       (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

   (2) INFORMATION FOR SEQ ID NO:29:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 24 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TCCGTAAGAA ACAGCCCAGT GACC   24

   (2) INFORMATION FOR SEQ ID NO:30:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 31 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCTCTGCGGC CGCTGTTGCA TTTCCTTTCT G   31

   (2) INFORMATION FOR SEQ ID NO:31:

     (i) SEQUENCE CHARACTERISTICS:

11

        (A) LENGTH: 19 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

      Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His
      1   5   10   15

      Gln Leu Leu

    (2) INFORMATION FOR SEQ ID NO:32:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

    TCCCTTGCCC TGACCACTCT T   21

    (2) INFORMATION FOR SEQ ID NO:33:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

    CCTCTGCGGC CGCACACACG TTGTCATGTG TTGC   34

    (2) INFORMATION FOR SEQ ID NO:34:

12

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 21 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TCCCTTGCCC TGACCACTCT T   21

(2) INFORMATION FOR SEQ ID NO:35:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 34 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCTCTGCGGC CGCCTTTTGC GTGGCTTCTC TGTT   34

(2) INFORMATION FOR SEQ ID NO:36:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 37 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA.

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCTCTGAGCT CAAGCTTGGT TTCCGGGGAC CACAATG   37

(2) INFORMATION FOR SEQ ID NO:37:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs

13

```
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA



      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

   CCTCTGCGGC CGCTAAGCAG CTTATTTTTA CTGAATGG                    38

   (2) INFORMATION FOR SEQ ID NO:38:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 37 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA



      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

   CCTCTGAGCT CAAGCTTGGT TTCCGGGGAC CACAATG                     37

   (2) INFORMATION FOR SEQ ID NO:39:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 33 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA



      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

   CCTCTGCGGC CGCCAGGGTA ACATCTATTC CAC                         33

   (2) INFORMATION FOR SEQ ID NO:40:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 35 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
```

14

      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCGAAGCTTC CACCATGAAC AAGTGGCTGT GCTGC   35

(2) INFORMATION FOR SEQ ID NO:41:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 40 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG   40

(2) INFORMATION FOR SEQ ID NO:42:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 21 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TCCCTTGCCC TGACCACTCT T   21

(2) INFORMATION FOR SEQ ID NO:43:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 35 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CCTCTGTCGA CTTAACACAC GTTGTCATGT GTTGC   35

(2) INFORMATION FOR SEQ ID NO:44:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 21 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TCCCTTGCCC TGACCACTCT T   21

(2) INFORMATION FOR SEQ ID NO:45:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 35 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CCTCTGTCGA CTTACTTTTG CGTGGCTTCT CTGTT   35

(2) INFORMATION FOR SEQ ID NO:46:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1537 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

16

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GTGAAGAGCG TGAAGAGCGG TTCCTCCTTT CAGCAAAAAA CCCCTCAAGA CCCGTTTAGA
60

GGCCCCAAGG GGTTATGCTA GTTATTGCTC AGCGGTGGCA GCAGCCAACT CAGCTTCCTT
120

TCGGGCTTTC TTCTTCTTCT TCTTCTTTCC GCGGATCCTC GAGTAAGCTT CCATGGTACC
180

CTGCAGGTCG ACACTAGTGA GCTCGAATTC CAACGCGTTA ACCATATGTT ATTCCTCCTT
240

TAATTAGTTA AAACAAATCT AGAATCAAAT CGATTAATCG ACTATAACAA ACCATTTTCT
300

TGCGTAAACC TGTACGATCC TACAGGTACT TATGTTAAAC AATTGTATTT CAAGCGATAT
360

AATAGTGTGA CAAAAATCCA ATTTATTAGA ATCAAATGTC AATCTATTAC CGTTTTAATG
420

ATATATAACA CGCAAAACTT GCGACAAACA ATAGGTAAGG ATAAAGAGAT GGGTATGAAA
480

GACATAAATG CCGACGACAC TTACAGAATA ATTAATAAAA TTAAAGCCTG TAGAAGCAAT
540

AATGATATTA ATCAATGCTT ATCTGATATG ACTAAAATGG TACATTGTGA ATATTATTTA
600

CTCGCGATCA TTTATCCTCA TTCTATGGTT AAATCTGATA TTTCAATTCT GGATAATTAC
660

CCTAAAAAAT GGAGGCAATA TTATGATGAC GCTAATTTAA TAAAATATGA TCCTATAGTA
720

GATTATTCTA ACTCCAATCA TTCACCGATT AATTGGAATA TATTTGAAAA CAATGCTGTA
780

AATAAAAAAT CTCCAAATGT AATTAAAGAA GCGAAATCAT CAGGTCTTAT CACTGGGTTT
840

AGTTTCCCTA TTCATACTGC TAATAATGGC TTCGGAATGC TTAGTTTTGC ACATTCAGAG
900

AAAGACAACT ATATAGATAG TTTATTTTTA CATGCGTGTA TGAACATACC ATTAATTGTT
960

CCTTCTCTAG TTGATAATTA TCGAAAAATA AATATAGCAA ATAATAAATC AAACAACGAT
1020

17

TTAACCAAAA GAGAAAAAGA ATGTTTAGCG TGGGCATGCG AAGGAAAAAG CTCTTGGGAT
1080

ATTTCAAAAA TATTAGGCTG TAGTAAGCGC ACGGTCACTT TCCATTTAAC CAATGCGCAA
1140

ATGAAACTCA ATACAACAAA CCGCTGCCAA AGTATTTCTA AAGCAATTTT AACAGGAGCA
1200

ATTGATTGCC CATACTTTAA AAGTTAAGTA CGACGTCCAT ATTTGAATGT ATTTAGAAAA
1260

ATAAACAAAA GAGTTTGTAG AAACGCAAAA AGGCCATCCG TCAGGATGGC CTTCTGCTTA
1320

ATTTGATGCC TGGCAGTTTA TGGCGGGCGT CCTGCCCGCC ACCCTCCGGG CCGTTGCTTC
1380

GCAACGTTCA AATCCGCTCC CGGCGGATTT GTCCTACTCA GGAGAGCGTT CACCGACAAA
1440

CAACAGATAA AACGAAAGGC CCAGTCTTTC GACTGAGCCT TTCGTTTTAT TTGATGCCTG
1500

GCAGTTCCCT ACTCTCGCAT GGGGAGACCA TGCATAC   1537

(2) INFORMATION FOR SEQ ID NO:47:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 48 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCCA   48

(2) INFORMATION FOR SEQ ID NO:48:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 55 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CGATTTGATT CTAGAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GGTAC
55

(2) INFORMATION FOR SEQ ID NO:49:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 49 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGAATTCCAA CGCGTTAACC ATATGTTATT CCTCCTTCTA GAATCAAAT   49

(2) INFORMATION FOR SEQ ID NO:50:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 1546 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCGTAACGTA TGCATGGTCT CCCCATGCGA GAGTAGGGAA CTGCCAGGCA TCAAATAAAA
60

CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTTTGTC GGTGAACGCT
120

CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG TTGCGAAGCA ACGGCCCGGA
180

GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC AAATTAAGCA GAAGGCCATC
240

19

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CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTTTGT TTATTTTTCT AAATACATTC
300

AAATATGGAC GTCGTACTTA ACTTTTAAAG TATGGGCAAT CAATTGCTCC TGTTAAAATT
360

GCTTTAGAAA TACTTTGGCA GCGGTTTGTT GTATTGAGTT TCATTTGCGC ATTGGTTAAA
420

TGGAAAGTGA CCGTGCGCTT ACTACAGCCT AATATTTTTG AAATATCCCA AGAGCTTTTT
480

CCTTCGCATG CCCACGCTAA ACATTCTTTT TCTCTTTTGG TTAAATCGTT GTTTGATTTA
540

TTATTTGCTA TATTTATTTT TCGATAATTA TCAACTAGAG AAGGAACAAT TAATGGTATG
600

TTCATACACG CATGTAAAAA TAAACTATCT ATATAGTTGT CTTTCTCTGA ATGTGCAAAA
660

CTAAGCATTC CGAAGCCATT ATTAGCAGTA TGAATAGGGA AACTAAACCC AGTGATAAGA
720

CCTGATGATT TCGCTTCTTT AATTACATTT GGAGATTTTT TATTTACAGC ATTGTTTTCA
780

AATATATTCC AATTAATCGG TGAATGATTG GAGTTAGAAT AATCTACTAT AGGATCATAT
840

TTTATTAAAT TAGCGTCATC ATAATATTGC CTCCATTTTT TAGGGTAATT ATCCAGAATT
900

GAAATATCAG ATTTAACCAT AGAATGAGGA TAAATGATCG CGAGTAAATA ATATTCACAA
960

TGTACCATTT TAGTCATATC AGATAAGCAT TGATTAATAT CATTATTGCT TCTACAGGCT
1020

TTAATTTTAT TAATTATTCT GTAAGTGTCG TCGGCATTTA TGTCTTTCAT ACCCATCTCT
1080

TTATCCTTAC CTATTGTTTG TCGCAAGTTT TGCGTGTTAT ATATCATTAA AACGGTAATA
1140

GATTGACATT TGATTCTAAT AAATTGGATT TTTGTCACAC TATTATATCG CTTGAAATAC
1200

AATTGTTTAA CATAAGTACC TGTAGGATCG TACAGGTTTA CGCAAGAAAA TGGTTTGTTA
1260

TAGTCGATTA ATCGATTTGA TTCTAGATTT GTTTTAACTA ATTAAAGGAG GAATAACATA
1320
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20

TGGTTAACGC GTTGGAATTC GAGCTCACTA GTGTCGACCT GCAGGGTACC ATGGAAGCTT
1380

ACTCGAGGAT CCGCGGAAAG AAGAAGAAGA AGAAGAAAGC CCGAAAGGAA GCTGAGTTGG
1440

CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG GGCCTCTAAA CGGGTCTTGA
1500

GGGGTTTTTT GCTGAAAGGA GGAACCGCTC TTCACGCTCT TCACGC   1546

(2) INFORMATION FOR SEQ ID NO:51:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 47 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TATGAAACAT CATCACCATC ACCATCATGC TAGCGTTAAC GCGTTGG   47

(2) INFORMATION FOR SEQ ID NO:52:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 49 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AATTCCAACG CGTTAACGCT AGCATGATGG TGATGGTGAT GATGTTTCA   49

(2) INFORMATION FOR SEQ ID NO:53:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 141 base pairs
         (B) TYPE: nucleic acid

21

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        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA



     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

 CTAATTCCGC TCTCACCTAC CAAACAATGC CCCCCTGCAA AAAATAAATT CATATAAAAA
 60

 ACATACAGAT AACCATCTGC GGTGATAAAT TATCTCTGGC GGTGTTGACA TAAATACCAC
 120

 TGGCGGTGAT ACTGAGCACA T                              141

  (2) INFORMATION FOR SEQ ID NO:54:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 147 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA



     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

 CGATGTGCTC AGTATCACCG CCAGTGGTAT TTATGTCAAC ACCGCCAGAG ATAATTTATC
 60

 ACCGCAGATG GTTATCTGTA TGTTTTTTAT ATGAATTTAT TTTTTGCAGG GGGGCATTGT
 120

 TTGGTAGGTG AGAGCGGAAT TAGACGT                        147

  (2) INFORMATION FOR SEQ ID NO:55:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 55 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA
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22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGATTTGATT CTAGAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GGTAC
55

(2) INFORMATION FOR SEQ ID NO:56:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 49 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CGAATTCCAA CGCGTTAACC ATATGTTATT CCTCCTTCTA GAATCAAAT   49

(2) INFORMATION FOR SEQ ID NO:57:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 668 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GTGAAGAGCG TGAAGAGCGG TTCCTCCTTT CAGCAAAAAA CCCCTCAAGA CCCGTTTAGA
60

GGCCCCAAGG GGTTATGCTA GTTATTGCTC AGCGGTGGCA GCAGCCAACT CAGCTTCCTT
120

TCGGGCTTTC TTCTTCTTCT TCTTCTTTCC GCGGATCCTC GAGTAAGCTT CCATGGTACC
180

CTGCAGGTCG ACACTAGTGA GCTCGAATTC CAACGCGTTA ACCATATGTT ATTCCTCCTT
240

23

TAATTAGTTA ACTCAAATCT AGAATCAAAT CGATAAATTG TGAGCGCTCA CAATTGAGAA
300

TAȚTAATCAA GAATTTTAGC ATTTGTCAAA TGAATTTTTT AAAAATTATG AGACGTCCAȚ
360

ATTTGAATGT ATTTAGAAAA ATAAACAAAA GAGTTTGTAG AAACGCAAAA AGGCCATCCG
420

TCAGGATGGC CTTCTGCTTA ATTTGATGCC TGGCAGTTTA TGGCGGGCGT CCTGCCCGCC
480

ACCCTCCGGG CCGTTGCTTC GCAACGTTCA AATCCGCTCC CGGCGGATTT GTCCTACTCA
540

GGAGAGCGTT CACCGACAAA CAACAGATAA AACGAAAGGC CCAGTCTTTC GACTGAGCCT
600

TTCGTTTTAT TTGATGCCTG GCAGTTCCCT ACTCTCGCAT GGGGAGACCA TGCATACGTT
660

ACGCACGT   668

(2) INFORMATION FOR SEQ ID NO:58:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 726 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GCGTAACGTA TGCATGGTCT CCCCATGCGA GAGTAGGGAA CTGCCAGGCA TCAAATAAAA
60

CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTTTGTC GGTGAACGCT
120

CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG TTGCGAAGCA ACGGCCCGGA
180

GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC AAATTAAGCA GAAGGGGCCT
240

CCCACCGCCC GTCCTGCGGG CGGTATTTGA CGGTCCGTAG TTTAATTCGT CTTCGCCATC
300

CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTTTGT TTATTTTTCT AAATACATTC
360

AAATATGGAC GTCTCATAAT TTTTAAAAAA TTCATTTGAC AAATGCTAAA ATTCTTGATT
420

AATATTCTCA ATTGTGAGCG CTCACAATTT ATCGATTTGA TTCTAGATTT GTTTTAACTA
480

ATTAAAGGAG GAATAACATA TGGTTAACGC GTTGGAATTC GAGCTCACTA GTGTCGACCT
540

GCAGGGTACC ATGGAAGCTT ACTCGAGGAT CCGCGGAAAG AAGAAGAAGA AGAAGAAAGC
600

CCGAAAGGAA GCTGAGTTGG CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG
660

GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA GGAACCGCTC TTCACGCTCT
720

TCACGC   726

(2) INFORMATION FOR SEQ ID NO:59:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 44 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TACGCCACTGG ATCCTTATAA GCAGCTTATT TTTACTGATT GGAC   44

(2) INFORMATION FOR SEQ ID NO:60:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GTCCTCCTGG TACCTACCTA AAACAAC   27

(2) INFORMATION FOR SEQ ID NO:61:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 102 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TATGGATGAA GAAACTTCTC ATCAGCTGCT GTGTGATAAA TGTCCGCCGG GTACCCGGCG
60

GACATTTATC ACACAGCAGC TGATGAGAAG TTTCTTCATC CA   102

(2) INFORMATION FOR SEQ ID NO:62:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 19 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

    Met Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro
    1   5   10   15

    Gly Thr Tyr

(2) INFORMATION FOR SEQ ID NO:63:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 84 base pairs
       (B) TYPE: nucleic acid

26

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    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA
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  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TATGGAAACT TTTCCTCCAA AATATCTTCA TTATGATGAA GAAACTTCTC ATCAGCTGCT
60

GTGTGATAAA TGTCCGCCGG GTAC   84

(2) INFORMATION FOR SEQ ID NO:64:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 78 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCATAA TGAAGATATT
60

TTGGAGGAAA AGTTTCCA   78

(2) INFORMATION FOR SEQ ID NO:65:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 44 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TACGCACTGG ATCCTTATAA GCAGCTTATT TTCACGGATT GAAC   44

  (2) INFORMATION FOR SEQ ID NO:66:

27

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 38 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GTGCTCCTGG TACCTACCTA AAACAGCACT GCACAGTG   38

(2) INFORMATION FOR SEQ ID NO:67:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 84 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TATGGAAACT CTGCCTCCAA AATACCTGCA TTACGATCCG GAAACTGGTC ATCAGCTGCT
60

GTGTGATAAA TGTGCTCCGG GTAC   84

(2) INFORMATION FOR SEQ ID NO:68:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 78 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT
60

28

TTGGAGGCAG AGTTTCCA   78

(2) INFORMATION FOR SEQ ID NO:69:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 54 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

TATGGACCCA GAAACTGGTC ATCAGCTGCT GTGTGATAAA TGTGCTCCGG GTAC
54

(2) INFORMATION FOR SEQ ID NO:70:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 48 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC TGGGTCCA   48

(2) INFORMATION FOR SEQ ID NO:71:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 87 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

29

TATGAAAGAA ACTCTGCCTC CAAAATACCT GCATTACGAT CCGGAAACTG GTCATCAGCT
60

GCTGTGTGAT AAATGTGCTC CGGGTAC   87

(2) INFORMATION FOR SEQ ID NO:72:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 81 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT
60

TTGGAGGCAG AGTTTCTTTC A   81

(2) INFORMATION FOR SEQ ID NO:73:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 71 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GTTCTCCTCA TATGAAACAT CATCACCATC ACCATCATGA AACTCTGCCT CCAAAATACC
60

TGCATTACGA T   71

  (2) INFORMATION FOR SEQ ID NO:74:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 base pairs
        (B) TYPE: nucleic acid

30

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        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA
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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GTTCTCCTCA TATGAAAGAA ACTCTGCCTC CAAAATACCT GCA   43

(2) INFORMATION FOR SEQ ID NO:75:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 76 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TACGCACTGG ATCCTTAATG ATGGTGATGG TGATGATGTA AGCAGCTTAT TTTCACGGAT
60

TGAACCTGAT TCCCTA   76

(2) INFORMATION FOR SEQ ID NO:76:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 47 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GTTCTCCTCA TATGAAATAC CTGCATTACG ATCCGGAAAC TGGTCAT   47

(2) INFORMATION FOR SEQ ID NO:77:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single

31

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GTTCTCCTAT TAATGAAATA TCTTCATTAT GATGAAGAAA CTT   43

(2) INFORMATION FOR SEQ ID NO:78:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 40 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TACGCACTGG ATCCTTATAA GCAGCTTATT TTTACTGATT   40

(2) INFORMATION FOR SEQ ID NO:79:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 40 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GTTCTCCTCA TATGGAAACT CTGCCTCCAA AATACCTGCA   40

(2) INFORMATION FOR SEQ ID NO:80:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 43 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

32

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TACGCACTGG ATCCTTATGT TGCATTTCCT TTCTGAATTA GCA   43

(2) INFORMATION FOR SEQ ID NO:81:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 18 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CCGGAAACAG ATAATGAG   18

(2) INFORMATION FOR SEQ ID NO:82:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 18 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GATCCTCATT ATCTGTTT   18

(2) INFORMATION FOR SEQ ID NO:83:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 30 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CCGGAAACAG AGAAGCCACG CAAAAGTAAG   30

(2) INFORMATION FOR SEQ ID NO:84:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 30 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GATCCTTACT TTTGCGTGGC TTCTCTGTTT   30

(2) INFORMATION FOR SEQ ID NO:85:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 12 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TATGTTAATG AG   12

(2) INFORMATION FOR SEQ ID NO:86:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 14 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GATCCTCATT AACA   14

(2) INFORMATION FOR SEQ ID NO:87:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 21 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TATGTTCCGG AAACAGTTAA G   21

(2) INFORMATION FOR SEQ ID NO:88:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 23 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GATCCTTAAC TGTTTCCGGA ACA   23

(2) INFORMATION FOR SEQ ID NO:89:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 36 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

35

TATGTTCCGG AAACAGTGAA TCAACTCAAA AATAAG   36

(2) INFORMATION FOR SEQ ID NO:90:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GATCCTTATT TTTGAGTTGA TTCACTGTTT CCGGAACA   38

(2) INFORMATION FOR SEQ ID NO:91:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 100 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CTAGCGACGA CGACGACAAA GAAACTCTGC CTCCAAAATA CCTGCATTAC GATCCGGAAA
60

CTGGTCATCA GCTGCTGTGT GATAAATGTG CTCCGGGTAC   100

(2) INFORMATION FOR SEQ ID NO:92:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 92 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

36

CCGGAGCACA TTTATCACAC AGCAGCTGAT GACCAGTTTC CGGATCGTAA TGCAGGTATT
60

TTGGAGGCAG AGTTTCTTTG TCGTCGTCGT CG   92

(2) INFORMATION FOR SEQ ID NO:93:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 26 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ACAAACACAA TCGATTTGAT ACTAGA   26

(2) INFORMATION FOR SEQ ID NO:94:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 50 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TTTGTTTTAA CTAATTAAAG GAGGAATAAA ATATGAGAGG ATCGCATCAC   50

(2) INFORMATION FOR SEQ ID NO:95:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 50 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CATCACCATC ACGAAACCTT CCCGCCGAAA TACCTGCACT ACGACGAAGA   50

(2) INFORMATION FOR SEQ ID NO:96:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 49 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

AACCTCCCAC CAGCTGCTGT GCGACAAATG CCCGCCGGGT ACCCAAACA   49

(2) INFORMATION FOR SEQ ID NO:97:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 26 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

TGTTTGGGTA CCCGGCGGGC ATTTGT   26

(2) INFORMATION FOR SEQ ID NO:98:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 50 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CGCACAGCAG CTGGTGGGAG GTTTCTTCGT CGTAGTGCAG GTATTTCGGC   50

(2) INFORMATION FOR SEQ ID NO:99:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 49 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GGGAAGGTTT CGTGATGGTG ATGGTGATGC GATCCTCTCA TATTTTATT   49

(2) INFORMATION FOR SEQ ID NO:100:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 50 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CCTCCTTTAA TTAGTTAAAA CAAATCTAGT ATCAAATCGA TTGTGTTTGT   50

(2) INFORMATION FOR SEQ ID NO:101:

   (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 59 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

39

ACAAACACAA TCGATTTGAT ACTAGATTTG TTTTAACTAA TTAAAGGAGG AATAAAATG
59

(2) INFORMATION FOR SEQ ID NO:102:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 48 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CTAATTAAAG GAGGAATAAA ATGAAAGAAA CTTTTCCTCC AAAATATC   48

(2) INFORMATION FOR SEQ ID NO:103:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 31 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TGTTTGGGTA CCCGGCGGAC ATTTATCACA C   31

(2) INFORMATION FOR SEQ ID NO:104:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 59 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

40

ACAAACACAA TCGATTTGAT ACTAGATTTG TTTTAACTAA TTAAAGGAGG AATAAAATG
59

(2) INFORMATION FOR SEQ ID NO:105:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 54 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CTAATTAAAG GAGGAATAAA ATGAAAAAAA AAGAAACTTT TCCTCCAAAA TATC
54

(2) INFORMATION FOR SEQ ID NO:106:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 31 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TGTTTGGGTA CCCGGCGGAC ATTTATCACA C   31

(2) INFORMATION FOR SEQ ID NO:107:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 44 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

41

CAGCCCGGGT AAAATGGAAA CGTTTCCTCC AAAATATCTT CATT   44

(2) INFORMATION FOR SEQ ID NO:108:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 44 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

CGTTTCCATT TTACCCGGGC TGAGCGAGAG GCTCTTCTGC GTGT   44

(2) INFORMATION FOR SEQ ID NO:109:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 45 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

CGCTCAGCCC GGGTAAAATG GAAACGTTGC CTCCAAAATA CCTGC   45

(2) INFORMATION FOR SEQ ID NO:110:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 39 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

CCATTTTACC CGGGCTGAGC GAGAGGCTCT TCTGCGTGT   39

42

(2) INFORMATION FOR SEQ ID NO:111:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 36 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

GAAAATAAGC TGCTTAGCTG CAGCTGAACC AAAATC   36

(2) INFORMATION FOR SEQ ID NO:112:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 34 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

CAGCTGCAGC TAAGCAGCTT ATTTTCACGG ATTG   34

(2) INFORMATION FOR SEQ ID NO:113:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 36 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

AAAAATAAGC TGCTTAGCTG CAGCTGAACC AAAATC   36

(2) INFORMATION FOR SEQ ID NO:114:

43

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 35 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

CAGCTGCAGC TAAGCAGCTT ATTTTTACTG ATTGG   35

(2) INFORMATION FOR SEQ ID NO:115:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 102 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

CTAGAAGGAG GAATAACATA TGGAAACTTT TGCTCCAAAA TATCTTCATT ATGATGAAGA
60

AACTAGTCAT CAGCTGCTGT GTGATAAATG TCCGCCGGGT AC   102

(2) INFORMATION FOR SEQ ID NO:116:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 94 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

44

CCGGCGGACA TTTATCACAC AGCAGCTGAT GACTAGTTTC TTCATCATAA TGAAGATATT
60

TTGGAGCAAA AGTTTCCATA TGTTATTCCT CCTT   94

(2) INFORMATION FOR SEQ ID NO:117:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 62 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

CTAGAAGGAG GAATAACATA TGGAAACTTT TCCTGCTAAA TATCTTCATT ATGATGAAGA
60

AA   62

(2) INFORMATION FOR SEQ ID NO:118:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 62 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

CTAGTTTCTT CATCATAATG AAGATATTTA GCAGGAAAAG TTTCCATATG TTATTCCTCC
60

TT   62

(2) INFORMATION FOR SEQ ID NO:119:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 51 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

Tyr His Tyr Tyr Asp Gln Asn Gly Arg Met Cys Glu Glu Cys His Met
1   5   10   15

Cys Gln Pro Gly His Phe Leu Val Lys His Cys Lys Gln Pro Lys Arg
        20   25   30

Asp Thr Val Cys His Lys Pro Cys Glu Pro Gly Val Thr Tyr Thr Asp
        35   40   45

Asp Trp His
    50

(2) INFORMATION FOR SEQ ID NO:120:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 2432 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 124..1326

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

ATCAAAGGCA GGGCATACTT CCTGTTGCCC AGACCTTATA TAAAACGTCA TGTTCGCCTG
60

GGCAGCAGAG AAGCACCTAG CACTGGCCCA GCGGCTGCCG CCTGAGGTTT CCAGAGGACC
120

ACA ATG AAC AAG TGG CTG TGC TGT GCA CTC CTG GTG TTC TTG GAC ATC
168
  Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile
    1   5   10   15

46

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ATT GAA TGG ACA ACC CAG GAA ACC TTT CCT CCA AAA TAC TTG CAT TAT
216
Ile Glu Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr
         20          25          30


GAC CCA GAA ACC GGA CGT CAG CTC TTG TGT GAC AAA TGT GCT CCT GGC
264
Asp Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly
         35          40          45


ACC TAC CTA AAA CAG CAC TGC ACA GTC AGG AGG AAG ACA CTG TGT GTC
312
Thr Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val
      50          55          60


CCT TGC CCT GAC TAC TCT TAT ACA GAC AGC TGG CAC ACG AGT GAT GAA
360
Pro Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu
      65          70          75


TGC GTG TAC TGC AGC CCC GTG TGC AAG GAA CTG CAG ACC GTG AAA CAG
408
Cys Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln
      80          85          90          95


GAG TGC AAC CGC ACC CAC AAC CGA GTG TGC GAA TGT GAG GAA GGG CGC
456
Glu Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg
         100         105         110


TAC CTG GAG CTC GAA TTC TGC TTG AAG CAC CGG AGC TGT CCC CCA GGC
504
Tyr Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly
         115         120         125


TTG GGT GTG CTG CAG GCT GGG ACC CCA GAG CGA AAC ACG GTT TGC AAA
552
Leu Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys
      130         135         140


AGA TGT CCG GAT GGG TTC TTC TCA GGT GAG ACG TCA TCG AAA GCA CCC
600
Arg Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro
   145         150         155


TGT AGG AAA CAC ACC AAC TGC AGC TCA CTT GGC CTC CTG CTA ATT CAG
648
Cys Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln
160         165         170         175
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47

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AAA GGA AAT GCA ACA CAT GAC AAT GTA TGT TCC GGA AAC AGA GAA GCA
696
Lys Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala
        180            185            190

ACT CAA AAT TGT GGA ATA GAT GTC ACC CTG TGC GAA GAG GCA TTC TTC
744
Thr Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe
        195            200            205

AGG TTT GCT GTG CCT ACC AAG ATT ATA CCG AAT TGG CTG AGT GTT CTG
792
Arg Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu
    210            215            220

GTG GAC AGT TTG CCT GGG ACC AAA GTG AAT GCA GAG AGT GTA GAG AGG
840
Val Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg
    225            230            235

ATA AAA CGG AGA CAC AGC TCG CAA GAG CAA ACT TTC CAG CTA CTT AAG
888
Ile Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys
240            245            250            255

CTG TGG AAG CAT CAA AAC AGA GAC CAG GAA ATG GTG AAG AAG ATC ATC
936
Leu Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile
        260            265            270

CAA GAC ATT GAC CTC TGT GAA AGC AGT GTG CAA CGG CAT ATC GGC CAC
984
Gln Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His
        275            280            285

GCG AAC CTC ACC ACA GAG CAG CTC CGC ATC TTG ATG GAG AGC TTG CCT
1032
Ala Asn Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro
    290            295            300

GGG AAG AAG ATC AGC CCA GAC GAG ATT GAG AGA ACG AGA AAG ACC TGC
1080
Gly Lys Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys
    305            310            315

AAA CCC AGC GAG CAG CTC CTG AAG CTA CTG AGC TTG TGG AGG ATC AAA
1128
Lys Pro Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys
320            325            330            335

AAT GGA GAC CAA GAC ACC TTG AAG GGC CTG ATG TAC GCA CTC AAG CAC
1176
Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His
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48

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        340            345          350
TTG AAA GCA TAC CAC TTT CCC AAA ACC GTC ACC CAC AGT CTG AGG AAG
1224
Leu Lys Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys
      355            360          365
ACC ATC AGG TTC TTG CAC AGC TTC ACC ATG TAC CGA TTG TAT CAG AAA
1272
Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys
    370            375          380
CTC TTT CTA GAA ATG ATA GGG AAT CAG GTT CAA TCA GTG AAG ATA AGC
1320
Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser
  385            390          395
TGC TTA TAGTTAGGAA TGGTCACTGG GCTGTTTCTT CAGGATGGGC CAACACTGAT
1376
Cys Leu
400
GGAGCAGATG GCTGCTTCTC CGGCTCTTGA AATGGCAGTT GATTCCTTTC TCATCAGTTG
1436
GTGGGAATGA AGATCCTCCA GCCCAACACA CACACTGGGG AGTCTGAGTC AGGAGAGTGA
1496
GGCAGGCTAT TTGATAATTG TGCAAAGCTG CCAGGTGTAC ACCTAGAAAG TCAAGCACCC
1556
TGAGAAAGAG GATATTTTTA TAACCTCAAA CATAGGCCCT TTCCTTCCTC TCCTTATGGA
1616
TGAGTACTCA GAAGGCTTCT ACTATCTTCT GTGTCATCCC TAGATGAAGG CCTCTTTTAT
1676
TTATTTTTTT ATTCTTTTTT TCGGAGCTGG GGACCGAACC CAGGGCCTTG CGCTTGCGAG
1736
GCAAGTGCTC TACCACTGAG CTAAATCTCC AACCCCTGAA GGCCTCTTTC TTTCTGCCTC
1796
TGATAGTCTA TGACATTCTT TTTTCTACAA TTCGTATCAG GTGCACGAGC CTTATCCCAT
1856
TTGTAGGTTT CTAGGCAAGT TGACCGTTAG CTATTTTTCC CTCTGAAGAT TTGATTCGAG
1916
TTGCAGACTT GGCTAGACAA GCAGGGGTAG GTTATGGTAG TTTATTTAAC AGACTGCCAC
1976
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49

CAGGAGTCCA GTGTTTCTTG TTCCTCTGTA GTTGTACCTA AGCTGACTCC AAGTACATTT
2036

AGTATGAAAA ATAATCAACA AATTTTATTC CTTCTATCCA CATTGGCTAG CTTTGTTTCA
2096

GGGCACTAAA AGAAACTACT ATATGGAGAA AGAATTGATA TTGCCCCCAA CGTTCAACAA
2156

CCCAATAGTT TATCCAGCTG TCATGCCTGG TTCAGTGTCT ACTGACTATG CGCCCTCTTA
2216

TTACTGCATG CAGTAATTCA ACTGGAAATA GTAATAATAA TAATAGAAAT AAAATCTAGA
2276

CTCCATTGGA TCTCTCTGAA TATGGGAATA TCTAACTTAA GAAGCTTTGA GATTTCAGTT
2336

GTGTTAAAGG CTTTTATTAA AAAGCTGATG CTCTTCTGTA AAAGTTACTA ATATATCTGT
2396

AAGACTATTA CAGTATTGCT ATTTATATCC ATCCAG   2432

(2) INFORMATION FOR SEQ ID NO:121:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 401 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile Ile
 1   5   10   15

Glu Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp
        20   25   30

Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
     35   40   45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
   50   55   60

Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
   65   70   75   80

50

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln Glu
          85   90   95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
        100   105   110

Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Leu
     115   120   125

Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
    130   135   140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145   150   155   160

Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln Lys
          165   170   175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
        180   185   190

Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
     195   200   205

Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
    210   215   220

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225   230   235   240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
          245   250   255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
        260   265   270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His Ala
     275   280   285

Asn Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro Gly
    290   295   300

51

ys Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305   310   315   320

Pro Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
    325   330   335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
    340   345   350

Lys Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
    355   360   365

Ile Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
   370   375   380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385   390   395   400

Leu

(2) INFORMATION FOR SEQ ID NO:122:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1324 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 90..1292

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

CCTTATATAA ACGTCATGAT TGCCTGGGCT GCAGAGACGC ACCTAGCACT GACCCAGCGG
60

CTGCCTCCTG AGGTTTCCCG AGGACCACA ATG AAC AAG TGG CTG TGC TGC GCA
113
                Met Asn Lys Trp Leu Cys Cys Ala
                 1   5

CTC CTG GTG CTC CTG GAC ATC ATT GAA TGG ACA ACC CAG GAA ACC CTT
161
Leu Leu Val Leu Leu Asp Ile Ile Glu Trp Thr Thr Gln Glu Thr Leu
    10   15   20

52

```
CCT CCA AAG TAC TTG CAT TAT GAC CCA GAA ACT GGT CAT CAG CTC CTG
209
Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His Gln Leu Leu
 25          30          35          40

TGT GAC AAA TGT GCT CCT GGC ACC TAC CTA AAA CAG CAC TGC ACA GTG
257
Cys Asp Lys Cys Ala Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Val
         45          50          55

AGG AGG AAG ACA TTG TGT GTC CCT TGC CCT GAC CAC TCT TAT ACG GAC
305
Arg Arg Lys Thr Leu Cys Val Pro Cys Pro Asp His Ser Tyr Thr Asp
     60          65          70

AGC TGG CAC ACC AGT GAT GAG TGT GTG TAT TGC AGC CCA GTG TGC AAG
353
Ser Trp His Thr Ser Asp Glu Cys Val Tyr Cys Ser Pro Val Cys Lys
     75          80          85

GAA CTG CAG TCC GTG AAG CAG GAG TGC AAC CGC ACC CAC AAC CGA GTG
401
Glu Leu Gln Ser Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val
     90          95          100

TGT GAG TGT GAG GAA GGG CGT TAC CTG GAG ATC GAA TTC TGC TTG AAG
449
Cys Glu Cys Glu Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys
105          110          115          120

CAC CGG AGC TGT CCC CCG GGC TCC GGC GTG GTG CAA GCT GGA ACC CCA
497
His Arg Ser Cys Pro Pro Gly Ser Gly Val Val Gln Ala Gly Thr Pro
         125          130          135

GAG CGA AAC ACA GTT TGC AAA AAA TGT CCA GAT GGG TTC TTC TCA GGT
545
Glu Arg Asn Thr Val Cys Lys Lys Cys Pro Asp Gly Phe Phe Ser Gly
     140          145          150

GAG ACT TCA TCG AAA GCA CCC TGT ATA AAA CAC ACG AAC TGC AGC ACA
593
Glu Thr Ser Ser Lys Ala Pro Cys Ile Lys His Thr Asn Cys Ser Thr
     155          160          165

TTT GGC CTC CTG CTA ATT CAG AAA GGA AAT GCA ACA CAT GAC AAC GTG
641
Phe Gly Leu Leu Leu Ile Gln Lys Gly Asn Ala Thr His Asp Asn Val
   170          175          180

TGT TCC GGA AAC AGA GAA GCC ACG CAA AAG TGT GGA ATA GAT GTC ACC
689
Cys Ser Gly Asn Arg Glu Ala Thr Gln Lys Cys Gly Ile Asp Val Thr
```

53

185   190   195   200

CTG TGT GAA GAG GCC TTC TTC AGG TTT GCT GTT CCT ACC AAG ATT ATA
737
Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Ile Ile
          205   210   215

CCA AAT TGG CTG AGT GTT TTG GTG GAC AGT TTG CCT GGG ACC AAA GTG
785
Pro Asn Trp Leu Ser Val Leu Val Asp Ser Leu Pro Gly Thr Lys Val
          220   225   230

AAT GCC GAG AGT GTA GAG AGG ATA AAA CGG AGA CAC AGC TCA CAA GAG
833
Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Arg His Ser Ser Gln Glu
     235   240   245

CAA ACC TTC CAG CTG CTG AAG CTG TGG AAA CAT CAA AAC AGA GAC CAG
881
Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Arg Asp Gln
   250   255   260

GAA ATG GTG AAG AAG ATC ATC CAA GAC ATT GAC CTC TGT GAA AGC AGC
929
Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Ser Ser
265   270   275   280

GTG CAG CGG CAT CTC GGC CAC TCG AAC CTC ACC ACA GAG CAG CTT CTT
977
Val Gln Arg His Leu Gly His Ser Asn Leu Thr Thr Glu Gln Leu Leu
          285   290   295

GCC TTG ATG GAG AGC CTG CCT GGG AAG AAG ATC AGC CCA GAA GAG ATT
1025
Ala Leu Met Glu Ser Leu Pro Gly Lys Lys Ile Ser Pro Glu Glu Ile
          300   305   310

GAG AGA ACG AGA AAG ACC TGC AAA TCG AGC GAG CAG CTC CTG AAG CTA
1073
Glu Arg Thr Arg Lys Thr Cys Lys Ser Ser Glu Gln Leu Leu Lys Leu
     315   320   325

CTC AGT TTA TGG AGG ATC AAA AAT GGT GAC CAA GAC ACC TTG AAG GGC
1121
Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly
   330   335   340

CTG ATG TAT GCC CTC AAG CAC TTG AAA ACA TCC CAC TTT CCC AAA ACT
1169
Leu Met Tyr Ala Leu Lys His Leu Lys Thr Ser His Phe Pro Lys Thr
345   350   355   360

54

```
GTC ACC CAC AGT CTG AGG AAG ACC ATG AGG TTC CTG CAC AGC TTC ACA
1217
Val Thr His Ser Leu Arg Lys Thr Met Arg Phe Leu His Ser Phe Thr
        365             370             375

ATG TAC AGA CTG TAT CAG AAG CTC TTT TTA GAA ATG ATA GGG AAT CAG
1265
Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln
        380             385             390

GTT CAA TCC GTG AAA ATA AGC TGC TTA TAACTAGGAA TGGTCACTGG
1312
Val Gln Ser Val Lys Ile Ser Cys Leu
        395             400

GCTGTTTCTT CA                                        1324
```

(2) INFORMATION FOR SEQ ID NO:123:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 401 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

```
Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu Asp Ile Ile
1           5           10          15

Glu Trp Thr Thr Gln Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp
        20          25          30

Pro Glu Thr Gly His Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
        35          40          45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
    50          55          60

Cys Pro Asp His Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65          70          75          80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu
        85          90          95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
        100         105         110

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser
```

55

    115   120   125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys
   130   135   140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145   150   155   160

Ile Lys His Thr Asn Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys
          165   170   175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
        180   185   190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
    195   200   205

Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
   210   215   220

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225   230   235   240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
          245   250   255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
   260   265   270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser
    275   280   285

Asn Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly
   290   295   300

Lys Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305   310   315   320

Ser Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
          325   330   335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
        340   345   350

Lys Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
    355   360   365

Met Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
   370   375   380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385   390   395   400

56

Leu

(2) INFORMATION FOR SEQ ID NO:124:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 1355 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 94..1296

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GTATATATAA CGTGATGAGC GTACGGGTGC GGAGACGCAC CGGAGCGCTC GCCCAGCCGC
60

CGCTCCAAGC CCCTGAGGTT TCCGGGGACC ACA ATG AAC AAG TTG CTG TGC TGC
114
                  Met Asn Lys Leu Leu Cys Cys
                  1   5

GCG CTC GTG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG
162
Ala Leu Val Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr
   10   15   20

TTT CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG
210
Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu
    25   30   35

TTG TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA
258
Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr
  40   45   50   55

GCA AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA
306
Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr
        60   65   70

GAC AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC
354
Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys

57

```
            75              80              85

AAG GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC
402
Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg
        90              95              100

GTG TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG
450
Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu
    105             110             115

AAA CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT GGA ACC
498
Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr
120             125             130             135

CCA GAG CGA AAT ACA GTT TGC AAA AGA TGT CCA GAT GGG TTC TTC TCA
546
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser
            140             145             150


AAT GAG ACG TCA TCT AAA GCA CCC TGT AGA AAA CAC ACA AAT TGC AGT
594
Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser
        155             160             165

GTC TTT GGT CTC CTG CTA ACT CAG AAA GGA AAT GCA ACA CAC GAC AAC
642
Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn
    170             175             180

ATA TGT TCC GGA AAC AGT GAA TCA ACT CAA AAA TGT GGA ATA GAT GTT
690
Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val
    185             190             195

ACC CTG TGT GAG GAG GCA TTC TTC AGG TTT GCT GTT CCT ACA AAG TTT
738
Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe
200             205             210             215

ACG CCT AAC TGG CTT AGT GTC TTG GTA GAC AAT TTG CCT GGC ACC AAA
786
Thr Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys
            220             225             230

GTA AAC GCA GAG AGT GTA GAG AGG ATA AAA CGG CAA CAC AGC TCA CAA
834
Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln
        235             240             245
```

58

```
GAA CAG ACT TTC CAG CTG CTG AAG TTA TGG AAA CAT CAA AAC AAA GCC
882
Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Ala
    250         255         260

CAA GAT ATA GTC AAG AAG ATC ATC CAA GAT ATT GAC CTC TGT GAA AAC
930
Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn
  265         270         275

AGC GTG CAG CGG CAC ATT GGA CAT GCT AAC CTC ACC TTC GAG CAG CTT
978
Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu
280         285         290         295

CGT AGC TTG ATG GAA AGC TTA CCG GGA AAG AAA GTG GGA GCA GAA GAC
1026
Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp
        300         305         310

ATT GAA AAA ACA ATA AAG GCA TGC AAA CCC AGT GAC CAG ATC CTG AAG
1074
Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys
      315         320         325

CTG CTC AGT TTG TGG CGA ATA AAA AAT GGC GAC CAA GAC ACC TTG AAG
1122
Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys
      330         335         340

GGC CTA ATG CAC GCA CTA AAG CAC TCA AAG ACG TAC CAC TTT CCC AAA
1170
Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys
  345         350         355

ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC AGC TTC
1218
Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
360         365         370         375

ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA GGT AAC
1266
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn
        380         385         390

CAG GTC CAA TCA GTA AAA ATA AGC TGC TTA TAACTGGAAA TGGCCATTGA
1316
Gln Val Gln Ser Val Lys Ile Ser Cys Leu
      395         400

GCTGTTTCCT CACAATTGGC GAGATCCCAT GGATGATAA                    1355
```

59

(2) INFORMATION FOR SEQ ID NO:125:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 401 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser Ile
1   5   10   15

Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp
        20   25   30

Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr
        35   40   45

Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro
    50   55   60

Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65   70   75   80

Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu
            85   90   95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr
        100   105   110

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe
    115   120   125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
    130   135   140

Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys
145   150   155   160

Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys
        165   170   175

Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr
        180   185   190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
    195   200   205

Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val
    210   215   220

60

Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225   230   235   240

Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
     245   250   255

Trp Lys His Gln Asn Lys Ala Gln Asp Ile Val Lys Lys Ile Ile Gln
     260   265   270

Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala
   275   280   285

Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly
   290   295   300

Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys
305   310   315   320

Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
        325   330   335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser
        340   345   350

Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr
     355   360   365

Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu
   370   375   380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385   390   395   400

Leu

(2) INFORMATION FOR SEQ ID NO:126:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 139 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

   Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys
   1   5   10   15

61

```
Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro
         20            25            30

Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala
     35            40            45

Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys
    50            55            60

Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr
65            70            75            80

Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn
         85            90            95

Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His
        100           105           110

Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly
     115           120           125

Phe Phe Leu Arg Glu Asn Gly Glu Cys Val Ser Cys
    130           135
```

(2) INFORMATION FOR SEQ ID NO:127:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 48 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

CCGGCGGACA TTTATCACAC AGCAGCTGAT GAGAAGTTTC TTCATCCA   48

(2) INFORMATION FOR SEQ ID NO:128:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 219 amino acids

62

```
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```
Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1         5           10              15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
      20            25            30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
      35          40            45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
   50          55            60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65        70            75            80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
         85            90            95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
      100           105           110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
      115           120           125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
   130         135           140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145           150           155           160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
         165           170           175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
      180           185           190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
      195           200           205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr
   210           215
```

63

(2) INFORMATION FOR SEQ ID NO:129:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 280 amino acids
     (B) TYPE: amino acid
    (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

  Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
  1   5   10   15

  Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
      20   25   30

  His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
     35   40   45

  Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50   55   60

  Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
  65   70   75   80

  Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
      85   90   95

  Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
     100   105   110

  Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
     115   120   125

  Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130   135   140

  Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
  145   150   155   160

  Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
      165   170   175

  Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
      180   185   190

  Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
     195   200   205

64

```
Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
    210             215             220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225             230             235             240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
            245             250             255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
        260             265             270

Phe Ser Pro Thr Pro Gly Phe Thr
    275             280
```

(2) INFORMATION FOR SEQ ID NO:130:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 207 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

```
Met Leu Arg Leu Ile Ala Leu Leu Val Cys Val Val Tyr Val Tyr Gly
1           5           10              15

Asp Asp Val Pro Tyr Ser Ser Asn Gln Gly Lys Cys Gly Gly His Asp
        20              25.             30

Tyr Glu Lys Asp Gly Leu Cys Cys Ala Ser Cys His Pro Gly Phe Tyr
        35          40              45

Ala Ser Arg Leu Cys Gly Pro Gly Ser Asn Thr Val Cys Ser Pro Cys
    50          55              60

Glu Asp Gly Thr Phe Thr Ala Ser Thr Asn His Ala Pro Ala Cys Val
65              70              75              80

Ser Cys Arg Gly Pro Cys Thr Gly His Leu Ser Glu Ser Gln Pro Cys
            85              90              95
```

65

Asp Arg Thr His Asp Arg Val Cys Asn Cys Ser Thr Gly Asn Tyr Cys
        100   105   110

Leu Leu Lys Gly Gln Asn Gly Cys Arg Ile Cys Ala Pro Gln Thr Lys
        115   120   125

Cys Pro Ala Gly Tyr Gly Val Ser Gly His Thr Arg Ala Gly Asp Thr
    130   135   140

Leu Cys Glu Lys Cys Pro Pro His Thr Tyr Ser Asp Ser Leu Ser Pro
145   150   155   160

Thr Glu Arg Cys Gly Thr Ser Phe Asn Tyr Ile Ser Val Gly Phe Asn
            165   170   175

Leu Tyr Pro Val Asn Glu Thr Ser Cys Thr Thr Thr Ala Gly His Asn
        180   185   190

Glu Val Ile Lys Thr Lys Glu Phe Thr Val Thr Leu Asn Tyr Thr
        195   200   205

(2) INFORMATION FOR SEQ ID NO:131:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 227 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Met ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1   5   10   15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
        20   25   30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
    35   40   45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
    50   55   60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65   70   75   80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
            85   90   95

66

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
        100   105   110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
    115   120   125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130   135   140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145   150   155   160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
        165   170   175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
        180   185   190

Asn Ala Ser Arg Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
    195   200   205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210   215   220

Gln His Thr
225

(2) INFORMATION FOR SEQ ID NO:132:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 197 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Met Val Ser Leu Pro Arg Leu Cys Ala Leu Trp Gly Cys Leu Leu Thr
1   5   10   15

Ala Val His Leu Gly Gln Ile Cys Val Thr Cys Ser Asp Lys Gln Tyr Leu
        20   25   30

His Asp Gly Gln Cys Cys Asp Leu Cys Gln Pro Gly Ser Arg Leu Thr
        35   40   45

67

Ser His Cys Thr Ala Leu Glu Lys Thr Gln Cys His Pro Cys Asp Ser
    50   55   60

Gly Glu Phe Ser Ala Gln Trp Asn Arg Glu Ile Arg Cys His Gln His
65   70   75   80

Arg His Cys Glu Pro Asn Gln Gly Leu Arg Val Lys Lys Glu Gly Thr
        85   90   95

Ala Glu Ser Asp Thr Val Cys Thr Cys Lys Glu Gly Gln His Cys Thr
        100   105   110

Ser Lys Asp Cys Glu Ala Cys Ala Gln His Thr Pro Cys Ile Pro Gly
    115   120   125

Phe Gly Val Met Glu Met ala Thr Glu Thr Thr Asp Thr Val Cys His
    130   135   140

Pro Cys Pro Val Gly Phe Phe Ser Asn Gln Ser Ser Leu Phe Glu Lys
145   150   155   160

Cys Tyr Pro Trp Thr Ser Cys Glu Asp Lys Asn Leu Glu Val Leu Gln
        165   170   175

Lys Gly Thr Ser Gln Thr Asn Val Ile Cys Gly Leu Lys Ser Arg Met
        180   185   190

Arg Ala Leu Leu Val
    195

(2) INFORMATION FOR SEQ ID NO:133:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 208 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Phe Leu Asp Ile Ile
1   5   10   15

Glu Trp Thr Thr Gln Glu Gln Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp
        20   25   30

Pro Glu Thr Gly Arg Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
        35   40   45

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Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
    50   55   60

Cys Pro Asp Tyr Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65   70   75   80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Thr Val Lys Gln Glu
            85   90   95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
        100   105   110

Leu Glu Leu Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Leu
        115   120   125

Gly Val Leu Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
    130   135   140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145   150   155   160

Arg Lys His Thr Asn Cys Ser Ser Leu Gly Leu Leu Leu Ile Gln Lys
        165   170   175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
        180   185   190

Gln Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
        195   200   205

(2) INFORMATION FOR SEQ ID NO:134:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 224 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

    Met Gly Ala Gly Ala Thr Gly Arg Ala Met Asp Gly Pro Arg Leu Leu
    1   5   10   15

    Leu Leu Leu Leu Leu Gly Val Ser Leu Gly Gly Ala Lys Glu Ala Cys
            20   25   30

    Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn
        35   40   45

69

Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr Val Cys
    50   55   60

Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val Val Ser Ala Thr
65   70   75   80

Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser Met Ser
        85   90   95

Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly
        100   105   110

Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg Val Cys
        115   120   125

Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr
    130   135   140

Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala Asn His
145   150   155   160

Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln
        165   170   175

Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro
        180   185   190

Gly Arg Trp Ile Thr Arg Ser Thr Pro Pro Glu Gly Ser Asp Ser Thr
    195   200   205

Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln Asp Leu Ile
    210   215   220

(2) INFORMATION FOR SEQ ID NO:135:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 205 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

    Met Tyr Val Trp Val Gln Gln Pro Thr Ala Phe Leu Leu Leu Gly Leu
    1   5   10   15

    Ser Leu Gly Val Thr Val Lys Leu Asn Cys Val Lys Asp Thr Tyr Pro
            20   25   30

70

Ser Gly His Lys Cys Cys Arg Glu Cys Gln Pro Gly His Gly Met Val
      35   40   45

Ser Arg Cys Asp His Thr Arg Asp Thr Val Cys His Pro Cys Glu Pro
  50   55   60

Gly Phe Tyr Asn Glu Ala Val Asn Tyr Asp Thr Cys Lys Gln Cys Thr
65   70   75   80

Gln Cys Asn His Arg Ser Gly Ser Glu Leu Lys Gln Asn Cys Thr Pro
          85   90   95

Thr Glu Asp Thr Val Cys Gln Cys Arg Pro Gly Thr Gln Pro Arg Gln
      100   105   110

Asp Ser Ser His Lys Leu Gly Val Asp Cys Val Pro Cys Pro Pro Gly
      115   120   125

His Phe Ser Pro Gly Ser Asn Gln Ala Cys Lys Pro Trp Thr Asn Cys
      130   135   140

Thr Leu Ser Gly Lys Gln Ile Arg His Pro Ala Ser Asn Ser Leu Asp
145   150   155   160

Thr Val Cys Glu Asp Arg Ser Leu Leu Ala Thr Leu Leu Trp Glu Thr
          165   170   175

Gln Arg Thr Thr Phe Arg Pro Thr Thr Val Pro Ser Thr Thr Val Trp
          180   185   190

Pro Arg Thr Ser Gln Leu Pro Ser Thr Pro Thr Leu Val
      195   200   205

(2) INFORMATION FOR SEQ ID NO:136:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 191 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

    Met Gly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu Val
    1   5   10   15

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Gly Cys Glu Lys Val Gly Ala Val Gln Asn Ser Cys Asp Asn Cys Gln
     20              25              30

Pro Gly Thr Phe Cys Arg Lys Tyr Asn Pro Val Cys Lys Ser Cys Pro
     35          40              45

Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Asn Cys Asn Ile Cys
   50              55          60

Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr
65          70              75          80

His Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro
         85              90              95

Gln Cys Thr Arg Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr
        100             105             110

Lys Gln Gly Cys Lys Thr Cys Ser Leu Gly Thr Phe Asn Asp Gln Asn
       115             120             125

Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Arg
   130             135             140

Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro
145             150             155             160

Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu
         165             170             175

Gly Gly Pro Gly Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu
       180             185             190
```

(2) INFORMATION FOR SEQ ID NO:137:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 54 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

TATGGATGAA GAAACTTCTC ATCAGCTGCT GTGTGATAAA TGTCCGCCGG GTAC
54

(2) INFORMATION FOR SEQ ID NO:138:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 380 amino acids

72

        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

   Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp Pro Glu Thr Gly His
   1   5   10   15

   Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr Tyr Leu Lys Gln His
        20   25   30

   Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro Cys Pro Asp His Ser
        35   40   45

   Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Val Tyr Cys Ser Pro
     50   55   60

   Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu Cys Asn Arg Thr His
   65   70   75   80

   Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr Leu Glu Ile Glu Phe
              85   90   95

   Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser Gly Val Val Gln Ala
        100   105   110

   Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys Cys Pro Asp Gly Phe
        115   120   125

   Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys Ile Lys His Thr Asn
     130   135   140

   Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys Gly Asn Ala Thr His
   145   150   155   160

   Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr Gln Lys Cys Gly Ile
              165   170   175

   Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr
        180   185   190

   Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp Ser Leu Pro Gly
        195   200   205

   Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Arg His Ser
     210   215   220

   Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn

73

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225           230           235           240

Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
        245           250           255

Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn Leu Thr Thr Glu
        260           265           270

Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys Lys Ile Ser Pro
        275           280           285

Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Ser Ser Glu Gln Leu
    290           295           300

Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr
305           310           315           320

Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys Thr Ser His Phe
        325           330           335

Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Met Arg Phe Leu His
        340           345           350

Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
        355           360           365

Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
    370           375           380
```

(2) INFORMATION FOR SEQ ID NO:139:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 380 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

```
Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1           5           10           15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
        20           25           30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
        35           40           45
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Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
    50          55          60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65          70          75          80

Asn Arg Val Cys Glu Cys Lys Gly Gly Arg Tyr Leu Glu Ile Glu Phe
        85          90          95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
        100         105         110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
     115         120         125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
    130         135         140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
    145         150         155         160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
        165         170         175

Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr
        180         185         190

Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly
     195         200         205

Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser
    210         215         220

Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn
    225         230         235         240

Lys Ala Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
        245         250         255

Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu
        260         265         270

Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala
     275         280         285

Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile
     290         295         300

Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr
305          310         315         320

Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe
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75

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           325           330          335

   Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His
          340           345          350

   Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
          355           360          365

   Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
       370           375          380
```

(2) INFORMATION FOR SEQ ID NO:140:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 30 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

TGGACCACCC AGAAGTACCT TCATTATGAC   30

(2) INFORMATION FOR SEQ ID NO:141:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 30 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

GTCATAATGA AGGTACTTCT GGGTGGTCCA   30

(2) INFORMATION FOR SEQ ID NO:142:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 31 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

76

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GGACCACCCA GCTTCATTAT GACGAAGAAA C   31

  (2) INFORMATION FOR SEQ ID NO:143:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 31 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GTTTCTTCGT CATAATGAAG CTGGGTGGTC C   31

  (2) INFORMATION FOR SEQ ID NO:144:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 29 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

GTGGACCACC CAGGACGAAG AAACCTCTC   29

  (2) INFORMATION FOR SEQ ID NO:145:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 29 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GAGAGGTTTC TTCGTCCTGG GTGGTCCAC   29

(2) INFORMATION FOR SEQ ID NO:146:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 29 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

CGTTTCCTCC AAAGTTCCTT CATTATGAC   29

(2) INFORMATION FOR SEQ ID NO:147:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 29 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

GTCATAATGA AGGAACTTTG GAGGAAACG   29

(2) INFORMATION FOR SEQ ID NO:148:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 32 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

GGAAACGTTT CCTGCAAAGT ACCTTCATTA TG   32

(2) INFORMATION FOR SEQ ID NO:149:

78

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 32 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

CATAATGAAG GTACTTTGCA GGAAACGTTT CC   32

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 27 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

CACGCAAAAG TCGGGAATAG ATGTCAC   27

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 27 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

GTGACATCTA TTCCCGACTT TTGCGTG   27

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 25 base pairs
    (B) TYPE: nucleic acid

79

        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

CACCCTGTCG GAAGAGGCCT TCTTC   25

(2) INFORMATION FOR SEQ ID NO:153:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

GAAGAAGGCC TCTTCCGACA GGGTG   25

(2) INFORMATION FOR SEQ ID NO:154:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

TGACCTCTCG GAAAGCAGCG TGCA   24

    (2) INFORMATION FOR SEQ ID NO:155:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

80

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

TGCACGCTGC TTTCCGAGAG GTCA   24

(2) INFORMATION FOR SEQ ID NO:156:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 24 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

CCTCGAAATC GAGCGAGCAG CTCC   24

(2) INFORMATION FOR SEQ ID NO:157:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 25 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

CGATTTCGAG GTCTTTCTCG TTCTC   25

  (2) INFORMATION FOR SEQ ID NO:158:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 33 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

CCGTGAAAAT AAGCTCGTTA TAACTAGGAA TGG   33

(2) INFORMATION FOR SEQ ID NO:159:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 33 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

CCATTCCTAG TTATAACGAG CTTATTTTCA CGG   33

(2) INFORMATION FOR SEQ ID NO:160:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

(2) INFORMATION FOR SEQ ID NO:161:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 44 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

82

CCTCTCTCGA GTCAGGTGAC ATCTATTCCA CACTTTTGCG TGGC   44

(2) INFORMATION FOR SEQ ID NO:162:

   (i) SEQUENCE CHARACTERISTICS:
   ↖ (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

(2) INFORMATION FOR SEQ ID NO:163:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

CCTCTCTCGA GTCAAGGAAC AGCAAACCTG AAGAAGGC   38

(2) INFORMATION FOR SEQ ID NO:164:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear   ·

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

(2) INFORMATION FOR SEQ ID NO:165:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

CCTCTCTCGA GTCACTCTGT GGTGAGGTTC GAGTGGCC   38

(2) INFORMATION FOR SEQ ID NO:166:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

CCTCTGAGCT CAAGCTTCCG AGGACCACAA TGAACAAG   38

  (2) INFORMATION FOR SEQ ID NO:167:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

CCTCTCTCGA GTCAGGATGT TTTCAAGTGC TTGAGGGC   38

(2) INFORMATION FOR SEQ ID NO:168:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 16 amino acids

84

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

Met Lys His His His His His His His Ala Ser Val Asn Ala Leu Glu
1   5   10   15